A Thesis for the Degree of Doctor of Philosophy

Inactivation of Foodborne Pathogens in Low Water Activity Food by Thermal and Non-Thermal Treatments

가열 및 비가열 처리를 통한 저 수분활성도 식품 내에 존재하는 식중독 균의 제어 방법

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Abstract

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The objectives of this study were (i) to investigate the effect of thermal treatment on inactivation of foodborne pathogens in low water activity foods, (ii) to evaluate the antimicrobial effect of non-thermal treatment against foodborne pathogens in foods, and (iii) to evaluate the antimicrobial effect of the combination treatment of non-thermal and thermal treatments against foodborne pathogens in low water activity foods.

To investigate the effect of thermal treatment on inactivation of foodborne pathogens in low a_w foods, peanut butter inoculated with *Salmonella* Senftenberg, *Salmonella* Typhimurium and *Salmonella* Tennessee were treated with a 915 MHz microwave with 2, 4 and 6 kW. Then acid and peroxide values and color changes were determined after 5 min of microwave heating. Six kW 915 MHz microwave treatment for 5

min reduced these three *Salmonella* serovars by 3.24 to 4.26 log CFU/g. Four kW 915 MHz microwave processing for 5 min reduced these *Salmonella* serovars by 1.14 to 1.48 log CFU/g. Microwave treatment did not affect acid, peroxide, or color values of peanut butter.

To evaluate the influence of a_w of samples on the antimicrobial effect of 915 MHz microwave heating, peanut butter inoculated with *Escherichia coli* O157:H7, *S.* Typhimurium and *Listeria monocytogenes* in peanut butter (0.3, 0.4 and 0.5 a_w) were treated with a 915 MHz microwave with 2, 4, and 6 kW for up to 5 min. Six kW 915 MHz microwave treatment for 5 min reduced these three pathogens by 1.97 to > 5.17 log CFU/g. Four kW 915 MHz microwave processing for 5 min reduced these pathogens by 0.41 to 1.98 log CFU/g. Weibull and Log-Linear + Shoulder models were used to describe the survival curves of three pathogens because they exhibited shouldering behavior. t_d (decimal reduction time) and t_{5d} (the time required for a 5 log reduction) values were calculated based on the Weibull and Log-Linear + Shoulder models. Generally, increased a_w resulted in shorter t_{5d} values of pathogens, but not shorter t_d values.

To evaluate the influence of microwave frequency and scale of samples on the antimicrobial effect of microwave heating, peanut butter with 4 different scales (50, 100, 200 and 400 g) were treated with a 2,450 MHz or 915 MHz microwave up to center temperature reached 100°C. When peanut butter samples were treated with 2,450 MHz, increasing peanut butter scale resulted in slow temperature increasing. But, in the case of 915 MHz, increasing peanut butter scale did not affect center temperature increasing. *E. coli* O157:H7 and *S.* Typhimurium inoculated peanut butter were treated with 2,450 MHz for 160 s or 915 MHz for 90 s. A 2,450 MHz microwave heating resulted in different inactivation level at different site and large standard deviation level at same site. But, 915 MHz microwave heat treated peanut butter sample showed much faster inactivation of foodborne pathogens and there were no site dependence or large standard deviation at same position. Furthermore, 915 MHz microwave heating did not affect acid, peroxide and color values.

But microwave system did not suitable for almonds pasteurization. To find suitable control intervention for almonds, the effect of packaging type

on inactivation of foodborne pathogens by dry heat was investigated. Almonds inoculated with *Salmonella* Enteritidis PT 30, *S.* Typhimurium or *S.* Senftenberg were treated with dry heat under open, ambient-sealed or vacuum-sealed packaging to evaluate how the packaging type influences the antimicrobial effect of dry heat. Color changes were determined after 1 h of dry heating. *Salmonella* populations were reduced following this sequence: open < ambient sealed < vacuum-sealed heating. Subjecting the three types of packaged almonds to dry heat did not affect color values. Moisture contents of ambient-sealed and open heat treated samples were reduced significantly ($P < 0.05$).

To investigate the effect of non-thermal treatment on inactivation of foodborne pathogens in foods, the antimicrobial effect of the combined treatment of ozone and pH against three foodborne pathogens in apple juice has been evaluated. Apple juice (pH 3.0, 4.0 and 5.0) inoculated with the three pathogens were treated with gaseous ozone (3.0 l/min flow rate and 2.0-3.0 g/m^3) for up to 4 min. Ozone treatment (4 min) of pH 3.0 apple juice resulted in > 5.36 log CFU/ml reduction of *E. coli* O157:H7. Ozone

treatment of pH 4.0 and 5.0 apple juice for 4 min reduced this pathogen by 5.12 log CFU/ml and 1.86 log CFU/ml, respectively. The combination of low pH and ozone showed a great antimicrobial effect in apple juice. *S.* Typhimurium and *L. monocytogenes* showed a reduction trend similar to *E. coli* O157:H7. There were no significant changes of color values when apple juice was treated with ozone, except for *b* values. Among all ozone treated samples, there were no significant differences in total phenolic contents.

To increase the efficacy of hydrogen peroxide vapor, the antimicrobial effect of vacuumed hydrogen peroxide vapor was evaluated. Black and red pepper inoculated with *E. coli* O157:H7 and *S.* Typhimurium were subjected to 0.5 ml of 10 to 50% vacuumed hydrogen peroxide vapor for 1 min, and color change was evaluated after treatment. Pathogen populations decreased with increasing hydrogen peroxide concentration. Fifty percent vacuumed hydrogen peroxide vapor treatment decreased *E. coli* O157:H7 and *S.* Typhimurium populations in black pepper > 5.34 and 4.52 log CFU/g, respectively, and 3.01 and 2.36 log CFU/g for *E.* coli O157:H7 and *S.* Typhimurium in red pepper, respectively, without causing color change.

To investigate the effect of the combined treatment of thermal and non-thermal treatment on inactivation of foodborne pathogens in low a_w foods, the antimicrobial effect of the combined treatment of mild heat and ozone against two foodborne pathogens in apple juice concentrates has been evaluated. Four types of apple juice concentrates (12, 18, 36, 72 °Brix) inoculated with pathogens were subjected to ozone (3.0 l/min flow rate and 2.0-3.0 g/m^3 concentration) and heat treatment (25, 45, and 50°C) simultaneously for 20, 40 and 60 s. Heat treatment alone (25, 45, and 50°C) for 1 min reduced populations of *E. coli* O157:H7 by 0 to 4.18 log CFU/ml in four types of apple juice concentrates. The combination of ozone and heat treatment for 1 min at 25 and 45°C reduced *E. coli* O157:H7 by 0.93 to 3.87 log CFU/ml and below the detection limit (> 1.0 log CFU/ml) at 50°C. A similar tendency was observed for *S.* Typhimurium. In several instances, results showed a synergistic effect of ozone and heat treatment. Color values were not changed during ozone and heat treatment. In all ozone treated samples, the concentration of residual ozone was reduced to under acceptable levels (< 0.4 mg/l).

As another available hurdle combination, the efficacy of vacuum-sealed dry heat combined with vacuumed hydrogen peroxide vapor for decontaminating seeds was investigated. Alfalfa seeds inoculated with three pathogens were subjected to 30% 1 ml of vacuumed hydrogen peroxide vapor and vacuum or open-dry heat at 73°C. Distilled water was used as a control. Vacuum-dry heat effectively reduced three pathogens in alfalfa seeds but open-dry heat showed less inactivation of pathogens. Sequential treatment of 30% 1 ml vacuumed hydrogen peroxide vapor (1 min) + Vacuum-dry heat (2 h) reduced these three pathogens by under the detection limit (1.0 log CFU/g). Sequential treatment did not affect the germination of alfalfa seeds.

In conclusion, the results of this study are helpful for the food industry to control pathogens in foods, especially low water activity foods. Non-thermal, thermal or combination of non-thermal and thermal treatment may suggest alternatives to currently used decontamination methods without quality changes. Also, pathogens inactivation studies, focus on low water activity foods, could facilitate preventing foodborne outbreaks due to pathogen contaminated low water activity foods.

Keywords: Foodborne Pathogen, Inactivation, Low Water Activity Food, Thermal Treatment, Non-thermal Treatment, Combination Treatment

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Chapter I.

General introduction

I-1. Literature review

I-1.1. Low water activity foods outbreaks

Low water activity (a_w) foods have low water activity which can reduce the growth of bacteria. The most bacteria can-not grow at a_w as low as 0.87. *Staphylococcus aureus* is one of the most famous salt tolerant foodborne pathogen which can grow a_w 0.85 foods. In this study, foods with $a_w < 0.85$ are referred low a_w foods. Low a_w foods contain seeds, cereals, grains, chocolate, powdered infant formula, cocoa powder, tree nuts, dried fruit and vegetables, peanuts, peanut butter, egg powder, fermented dry sausage, pasta, flour, spices, honey, meat powder and milk powder. As I mentioned above, low a_w foods have low water activity which prevent growth of microorganism but low water content of low a_w foods reduce thermal efficiency. Low thermal efficiency of foods results in difficulty of pasteurization by conventional heating treatment. And also, quality deterioration can be occurred during pasteurization of low a_w foods by conventional heating due to long treatment time.

Moreover, there were many outbreaks due to pathogen contaminated

low a_w foods were have been reported. In 2007, there was a large outbreak in the USA caused by *Salmonella enterica* serovar Tennessee traced to contaminated peanut butter. Because of this outbreak, 425 people were infected with *S.* Tennessee resulting in 71 hospitalizations (CDC, 2007a). Between September 2008 and April 2009, there was a large multistate outbreak caused by *Salmonella* Typhimurium-contaminated peanut butter. This outbreak resulted in 714 illnesses, 166 hospitalizations and 9 deaths (Cavallaro et al., 2011). And, in 2012 in the USA, 42 cases of *Salmonella* Bredeney infections linked to peanut butter consumption were reported (CDC, 2012). Also, unfortunately, there have been several outbreaks due to *Salmonella* contaminated almonds. During 2000-2001 in Canada and the United States, there was a large outbreak of salmonellosis due to consumption of *Salmonella* Enteritidis PT 30-contaminated almonds. As a result of this outbreak 168 people became infected with *S.* Enteritidis PT 30 (Isaac et al., 2005). In 2003 to 2004, 29 cases of *S.* Enteritidis PT 30 infections linked to almonds consumption were reported (CDC, 2004). There was also an outbreak of *S.* Enteritidis PT 30 infections in Sweden in

2005 to 2006 linked to almonds (Müller et al., 2007). In every instance *S.* Enteritidis PT 30 was implicated. But, in 2009, there was an outbreak of *Salmonella* (including Montevideo, Newport and Senftenberg) contaminated pistachios (CDC, 2009). Also, *Salmonella* Typhimurium was detected in almonds (Danyluk et al., 2007). Moreover, pathogen-contaminated spices are wide spread. Choo et al. (2007) reported that 84.3% of dried red pepper samples purchased in Korea were contaminated with *Bacillus cereus*. Burow and Pudich (1996) reported that out of 317 spice samples, *Salmonella* was detected in 5.4% of them. Also, Little et al. (2003) reported that *Salmonella* and *B. cereus* were detected in dried spice mix. These high levels of microorganisms and pathogen contaminations have resulted in large outbreaks. In 2010 in the USA, there was a large outbreak of *Salmonella* Montevideo traced to contaminated sliced salami. Black and red pepper added to salami after the pasteurization step was the main source of pathogen contamination. Due to this outbreak 272 people in 44 states became ill (Gieraltowski et al., 2010). And also, there have been several multistate outbreaks due to seed sprouts after 2010 in the U.S. In 2011, there

was an outbreak due to consumption of *Salmonella* Enteritidis contaminated alfalfa and spicy seed sprouts (CDC, 2011). In 2014, an outbreak of *Escherichia coli* O121 linked to raw clover sprouts was reported in 6 states (CDC, 2014). And, in 2014, there was a large multistate outbreak of *S.* Enteritidis linked to bean sprouts resulting in 115 infections (CDC, 2015a). In 2016, there were three multistate outbreaks of *Salmonella* and *E. coli* O157:H7 linked to alfalfa sprouts and there was even an outbreak of sprouts contaminated with *Listeria* reported in 2014 (CDC, 2015b, 2016b, 2016c, 2016d). The most likely source of sprout-linked outbreaks has been seeds (NACMF, 1999a). Pathogens confined to seed surfaces or present on shoots during germination (NACMF, 1999b) can multiply rapidly during sprouting because the warm, humid conditions necessary for germination and sprout growth are also ideal for microbial growth (Hong and Kang, 2016). And there was one study which confirmed the survival of pathogens in juice of high solids content with $a_w < 0.85$ (Oyarzábal et al., 2003). Examples of outbreaks caused by these low a_w foods (peanut butter, nuts, spices and sprouts) were listed in Table I-1.

Table I-1. Outbreaks caused by low a_w foods (CDC, 2016a).

Year	Genus Species	Serotype/ Genotype	Total Ill	Total Hospitalization	Total Death	Food Vehicle
1999	*Salmonella enterica*	Typhimurium	112	3	0	Clover sprouts
1999	*Salmonella enterica*	Muenchen	157	16	0	Alfalfa sprouts
1999	*Salmonella enterica*	Enteritidis	108	13	0	Mung bean sprouts
2001	*Salmonella enterica*	Heidelberg	93	18	1	Peppers, green
2003	*Salmonella enterica*	Enteritidis	42	10	0	Nuts, almonds
2006	*Salmonella enterica*	Thompson	100	3	70	Peanuts
2006	*Salmonella enterica*	Tennessee	715	129	0	Peanut butter

Table I-1. (continued).

Year	Genus Species	Serotype/ Genotype	Total Ill	Total Hospitalization	Total Death	Food Vehicle
2008	*Salmonella enterica*	Typhimurium	714	166	9	Peanut butter, peanut paste
2008	*Salmonella enterica*	Saintpaul	1500	308	2	Peppers
2008	*Salmonella enterica*	Rissen	87	0	0	White pepper, ground
2009	*Salmonella enterica*	Saintpaul	256	8	0	Alfalfa sprouts
2010	*E. coli*	O157:H7	8	3	0	Nuts, hazelnuts
2010	*Salmonella enterica*	Newport	44	0	0	Alfalfa sprouts
2011	*Salmonella enterica*	Enteritidis	53	2	0	Turkish pine nuts

Table I-1. (continued).

Year	Genus Species	Serotype/ Genotype	Total Ill	Total Hospitalization	Total Death	Food Vehicle
2011	*E. coli*	O26	29	7	0	Clover sprouts
2012	*Salmonella enterica*	Bredeney	42	0	0	Peanut butter
2014	*Salmonella enterica*	Braenderup	6	1	0	Nut butter
2014	*Salmonella enterica*	Enteritidis	115	19	0	Mung bean sprouts
2014	*Bacillus cereus*		31	0	0	Peppers
2014	*E. coli*	O121	19	2	0	Clover sprouts
2015	*Salmonella enterica*	Java	13	0	0	Sprouted nut butter

I-1.2. Pasteurization of low water activity foods by conventional heating

Conventional heating is the most popular pasteurization intervention because it is the simplest control method to pasteurize foods. Table I-2 shows studies on the antimicrobial effect of conventional heating against pathogens in low a_w foods. It is important to consider the treatment type of conventional heating when comparing studies on inactivation of pathogen. The antimicrobial effect of conventional heating increase, decrease depending on the type of conventional heating and moisture contents during treatment.

Table I-2. Studies on the antimicrobial effect of conventional heating against pathogens in low a_w foods.

Sample	A_w	Pathogens	Treatment type	Treatment temperature	Treatment time	Log reduction	Reference
Peanut butter	0.45	*S.* Tennessee	Moist heat	71°C	50 min	2.00	Ma et al. (2009)
				77°C		2.89	
				83°C		3.69	
				90°C		5.13	
		S. enterica		71°C	50 min	2.16	
				77°C		3.29	
				83°C		3.60	
				90°C		4.41	
	0.5	*S. enterica*	Moist heat	70°C	5 min	1.4	Shachar and Yaron (2006)
				80°C		2.2	
				90°C		2.5	
				70°C	20 min	2.7	
				80°C		3.0	
				90°C		3.0	
				70°C	50 min	≈ 4.0	
				80°C		≈ 4.0	
				90°C		≈ 4.0	
	0.4	*E. coli* O157:H7	Moist heat	72°C	60 min	< 2.0	He et al. (2011)
				90°C		7.1	

Table I-2. (Continued).

Sample	A_w	Pathogens	Treatment type	Treatment temperature	Treatment time	Log reduction	Reference
Peanut butter	0.4	*S. enterica*	Moist heat	72°C	60 min	< 2.00	He et al. (2011)
				90°C		5.5	
Almonds	0.42	*S.* Enteritidis	Convective heat (dry roasting)	150°C	2.5 min	≈ 0	Jeong and Kang (2017)
					10 min	1.7	
		S. Typhimurium			2.5 min	≈ 0	
					10 min	2.5	
		S. Senftenberg			2.5 min	≈ 0	
					10 min	3.7	
	ND[a]	*S.* Enteritidis	Hot water	70°C	4 min	3.9	Harris et al. (2012)
	ND	*S.* Enteritidis	Saturated steam	100°C	45 s	> 5.0	Chang et al. (2012)
	ND	*S.* Enteritidis	Saturated steam	93°C	25 s	2.8	Lee et al. (2006)
	ND	*S.* Enteritidis	Moist-air convection heating (5%)	121°C	30 s	0.16	Jeong et al. (2009)
					180 s	0.87	
					300 s	1.16	

Table I-2. (Continued).

Sample	A_w	Pathogens	Treatment type	Treatment temperature	Treatment time	Log reduction	Reference
Almonds	ND	*S.* Enteritidis	Moist-air convection heating (5%)	121°C	1260 s	0.78	Jeong et al. (2009)
					1800 s	1.66	
			Moist-air convection heating (50%)	121°C	6 s	0.75	
					58 s	2.43	
					104 s	4.01	
					150 s	3.62	
Red pepper	11.40% (moisture content)	*E. coli* O157:H7	Dry heat	35°C	5 min	0.17	Cheon et al. (2015)
				45°C		0.33	
				55°C		0.88	
				65°C		1.23	
		S. Typhimurium		35°C		0.41	
				45°C		0.67	
				55°C		1.02	
				65°C		1.76	

Table I-2. (Continued).

Sample	A_w	Pathogens	Treatment type	Treatment temperature	Treatment time	Log reduction	Reference
Alfalfa seeds	0.439	*S.* Typhimurium	Dry heat	55°C	2 days	1.4	Neetoo and Chen (2011)
					4 days	1.8	
		E. coli O157:H7			2 days	2.9	
					4 days	5.1	
	0.22	*S.* Typhimurium		60°C	12 h	0.26	Hong and Kang (2016)
					18 h	0.32	
					24 h	0.71	
				70°C	12 h	1.37	
					18 h	1.39	
					24 h	1.45	
				80°C	12 h	1.78	
					18 h	2.20	
					24 h	2.76	
	ND	*S. enterica*		55°C	4 days	5.86	Feng et al. (2007)
					6 days	6.68	
		E. coli O157:H7			4 days	7.15	
					6 days	> 7.75	

[a] ND : non-defined.

I-1.3. Pasteurization of low water activity foods by various control methods

Most studies in Table I-2 used high temperature or/and long treatment time to reduce foodborne pathogens in low a_w foods. Conventional heating transfers heat by conduction or convection which leads to long treatment time due to slow heat transfer rate. Conventional heating of low a_w food is a time and energy intensive intervention. Moreover, food ingredients are heat sensitive which means that food quality deterioration can occur during conventional heating (Chemat et al., 2011). Excess thermal treatment results in quality deterioration of food such as color, flavor, texture (Lee et al., 2016). From these reasons, many researchers have been used various control methods to reduce foodborne pathogens in low a_w foods. Table I-3 shows current studies on the antimicrobial effect of various control methods against pathogenic microorganisms in low a_w foods.

Table I-3. Studies on the antimicrobial effect of various control methods against pathogens in low a_w foods.

Sample	A_w or moisture content	Pathogens	Treatment	Log reduction	Reference
Black pepper (ground)	10.4%	*E. coli*	Ozone treatment (concentration: 6.7 mg/l, flow rate: 6 l/min) for 60 min	≈ 3.0	Zhao and Cranston (1995)
		Salmonella		≈ 3.0	
(whole)	ND[a]	*Salmonella*	Direct plasma treatment with a radio frequency plasma jet for 15 min	2.0~3.0	Hertwig et al. (2015b)
Red pepper	ND	*E. coli*	Ozone treatment (1.0 ppm) for 360 min	1.7	Akbas and Ozdemir (2008)
		B. cereus		1.5	
	≈ 0.5	*B. cereus* (spore)	Microwave assisted cold plasma treatment (900 W) for 20 min	≈ 0	Kim et al. (2014)
	11.40%	*E. coli* O157:H7	Combination treatment of mild heat (65°C) and UV for 10 min	2.88	Cheon et al. (2015)
		S. Typhimurium		3.06	
	0.68	*E. coli* O157:H7	Combination treatment of NIR and UV for 5 min	2.78	Ha and Kang (2013)
		S. Typhimurium		3.34	
Peanut butter cracker sandwich	0.4	*E. coli* O157:H7	9 kW, 27.12 MHz radio frequency heating for 90 s	4.39	Ha et al. (2013)
		S. Typhimurium		4.29	

Table I-3. (Continued).

Sample	A_w or moisture content	Pathogens	Treatment	Log reduction	Reference
Almonds	ND	*E. coli* O157:H7	Cold plasma treatment (gas: air, frequency 47 kHz, distance 2, 4, 6 cm) for 20 s	≈ 1.0	Niemira (2012)
		S. enterica	405 nm high-intensity monochromatic blue light (3.4 W, distance 7 cm) for 10 min	≈ 1.0	Lacombe et al. (2016)
		E. coli O157:H7		1.69	
	5%	*S.* Enteritidis	Infrared heating for 45 s	1.51	Brandl et al. (2008)
Pistachios	ND	*E. coli*	Ozone treatment (1.0 ppm) for 360 min	3.5	Akbas and Ozdemir (2006)
		B. cereus		3	
	ND	*E. coli* O157:H7	70% ethanol and two quaternary ammonium sanitizers	≈ 0	McEgan and Danyluk (2015)
		Salmonella		≈ 0	
		L. monocytogenes		2~4	
Alfalfa seeds	ND	*E. coli*	4000 psi supercritical carbon dioxide for 60 min	1.0	Mazzoni et al. (2001)
	ND	*E. coli* O157:H7	21 ppm ozonated water for 64 min	1.49	Sharma et al. (2002)

Table I-3. (Continued).

Sample	A_w or moisture content	Pathogens	Treatment	Log reduction	Reference
Alfalfa seeds	ND	*Salmonella*	Electrolyzed oxidizing water (concentration of chlorine was 84 mg/l) for 10 min	1.65	Kim et al. (2003)
	ND	*E. coli* O157:H7	Pulsed UV for 90 s (270 pulses)	4.89	Sharma and Demirci (2003)
	ND	*S. enterica*	Ultrasound treatment (38.5-40.5 kH) for 5 min at 55°C	3.66	Scouten and Beuchat (2002)
	0.439	*Salmonella*	Combination treatment on dry heat (2 day at 55°C) and high pressure (600 MPa for 2 min at 20°C)	4.3	Neetoo and Chen (2011)
	0.22	*S.* Typhimurium	Sequential treatment of dry heat (24 h at 80°C) and hydrogen peroxide (2% for 10 min)	3.6	Hong and Kang (2016)
	ND	*E. coli* O157:H7 *S.* Enteritidis	Carvacrol emulsion (4000 ppm) for 60 min	3.8 3.9	Landry et al. (2014)

Table I-3. (Continued).

Sample	A_w or moisture content	Pathogens	Treatment	Log reduction	Reference
Radish seeds	ND	*E. coli* O157:H7	Combination treatment of chlorine dioxide (200 ppm for 5 min), drying (25°C for 2 h) and dry heat (55°C for 6 h)	4.8	Bang et al. (2011a)
	< 0.3	*E. coli* O157:H7	Combination treatment of chlorine dioxide (500 ppm for 5 min), drying (45°C for 24 h) and dry heat (70°C for 24 h)	> 4.9	Bang et al. (2011b)
Mung bean	ND	*E. coli* O157:H7	Aerated steam treatment at 70°C for 30 s	1.32	Studer et al. (2013)
		S. Weltevreden		1.5	
		L. monocytogenes		0.4	
Cantaloupe seeds	3-4%	*S.* Poona	Chlorine dioxide gas (10 mg/l) for 3 min at 75% relative humidity	2.02	Trinetta et al. (2011)
Tomato seeds	3-4%	*S.* Poona		5.32	
Lettuce seeds	3-4%	*S.* Poona		1.96	

[a] ND : non-defined.

I-2. Limitations of current studies on pasteurization of low a_w foods

Based on the literature review of current studies on the pasteurization of low a_w foods by various control methods, some limitations are identified. First of all, most non-thermal treatments were not effective on pasteurization of low a_w foods. Niemira (2012) used cold plasma to reduce pathogens on almonds. But 20 s of cold plasma treatment reduced *E. coli* O157:H7 and *Salmonella* only by about one log CFU/g. Lacombe et al. (2016) reported that 405 nm monochromatic blue light for 10 min reduced *S.* Enteritidis PT 30 by 0.5 to 1.26 log CFU/g. Akbas and Ozdemir (2006; 2008) used ozone to inactivate *E. coli* in pistachio and red pepper samples. One ppm of ozone for 360 min reduced *E. coli* by 3.5 and 1.7 log CFU/g, respectively. These results show that non-thermal treatment is too time-consuming to be economically feasible for reducing pathogens or is not effectively reduced pathogens on low a_w foods.

Secondly, aqueous sanitizer treatments used in previous studies were needed additional drying step. Bang et al. (2011a) reported that DH at 55°C for 12 h following 5 min of 500 ppm chlorine dioxide treatment and drying for 2 h at 25°C reduced *E. coli* O157:H7 on radish seeds to under the detection limit (0.8 log CFU/g). Also, Bang et al (2011b) reported that 500

ppm chlorine dioxide treatment for 5 min and drying for 24 h at 45°C reduced *E. coli* O157:H7 on radish seeds to under the detection limit (1.0 log CFU/g). And also, other aqueous sanitizer treatments (Hong and Kang, 2016; Kim et al., 2003; McEgan and Danyluk, 2015; Scouten and Beuchat, 2002; Sharma et al., 2002) also need additional drying step after pasteurization to reduce water content under microbiologically safe moisture level.

Thirdly, dry heat treatment required excessive time to reduce pathogens. Feng et al. (2007) and Neetoo and Chen (2011) used dry heat to reduce pathogens on seeds for several days. To reduce the long treatment time needed for dry heat treatment, investigators have experimented with combining chemicals and dry heat. But, the combination treatment of dry heat and sanitizer also required long treatment time. Hong and Kang (2016) used sequential treatment with dry heat and 2% hydrogen peroxide for 24 h and Bang et al. (2011a) used dry heat at 55°C for 12 h following 5 min of 500 ppm chlorine dioxide treatment and drying for 2 h at 25°C. Also, Bang et al (2011b) used 500 ppm chlorine dioxide treatment for 5 min and drying for 24 h at 45°C.

Lastly, there is no control method which is applicable to all kinds of low a_w foods because foods are extremely complex. Trinetta et al. (2011) reported that chlorine dioxide gas (10 mg/l) for 3 min reduced *S.* Poona on tomato seeds by 5.32 log CFU/g but the same treatment reduced the same

pathogen on lettuce seeds by 1.96 log CFU/g. Radio frequency (9 kW, 27.12 MHz) for 90 s reduced *S.* Typhimurium and *E. coli* O157:H7 in peanut butter cracker sandwiches by 4.29 and 4.39, respectively, but radio frequency heating did not increased the temperature of peanut butter (data not shown). Saturated steam (93°C, 25 s) reduced *S.* Enteritidis PT30 on almonds by 2.8 log CFU/g but steam treatment is only suitable for surface disinfection. And also, 915 MHz microwave heating effectively reduced *Salmonella* in peanut butter (Song and Kang, 2016a) but this system was not effectively reduce pathogens on almonds and surface burning was occurred after treatment (data not shown).

I-3. Objectives of this study

The specific objectives of this study were,

(1) to investigate the effect of thermal treatment (915 MHz microwave heating, vacuum-sealed dry heat) on inactivation of foodborne pathogens in low water activity foods

The effect of 915 MHz microwave heating on inactivation of *Salmonella* in peanut butter and the quality change of peanut butter were evaluated. Also, the effect of varying levels of a_w on the antimicrobial effect of 915 MH microwave heating on peanut butter. Lastly, this study determined how microwave treatment frequency and sample volume influence the antimicrobial effect of microwave heating against foodborne pathogens in peanut butter. Because 915 MHz microwave heating was not suitable for almonds pasteurization, the effect of vacuum-sealed dry heat treatment on the inactivation of *Salmonella* on almonds was measured.

(2) to evaluate the antimicrobial effect of non-thermal treatment against foodborne pathogens in foods. The effect of gaseous ozone treatment on the juice was examined to apply juice pasteurization. Also, this study examined the effect of vacuumed hydrogen peroxide vapor treatment on inactivation of *E. coli* O157:H7 and *S.* Typhimurium in black and red pepper. Vacuumed hydrogen peroxide vapor was also applied as a combination treatment to reduce pathogens on seeds.

(3) to evaluate the antimicrobial effect of the combination treatment of non-thermal and thermal treatments against foodborne pathogens in low water activity foods. This study examined how different soluble solid content (°Brix) of apple juice concentrate influence the antimicrobial effect of combination treatment of ozone and mild heat against foodborne pathogens in these selected conditions. Also, this study determined the combination effect of vacuumed hydrogen peroxide vapor and vacuum-sealed dry heat treatment against foodborne pathogens on seeds.

Chapter II.

Inactivation of foodborne pathogens in low A_w foods by thermal treatment

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II-1. Inactivation of *Salmonella* in peanut butter by 915 MHz microwave heating

II-1.1. Introduction

Non-typhoidal salmonellae are among the most important foodborne pathogens and can cause gastroenteritis, bacteremia and subsequent focal infection (Hohmann, 2001). Scallan et al. (2011) estimated there are over 9 million foodborne illnesses in the United States annually; about a million of these cases are caused by non-typhoidal *Salmonella* spp. Based on this estimate, about twenty thousand people are hospitalized and 378 die annually due to non-typhoidal *Salmonella* spp. People believe that low water activity (a_w) foods are free from contamination with *Salmonella* because the optimum a_w for growth of *Salmonella* is 0.99 (Mattick et al., 2000). However, several incidents of *Salmonella* contamination of low a_w foods have been reported. In 2001, there was an international outbreak caused by multi-resistance *Salmonella enterica* serovar Typhimurium DT 104 in Australia and Sweden. The main source of contamination was traced to halvah (a candy made of sesame seed, sugar and flavoring) imported from Turkey (Brockmann et al., 2004). In 1993, the US Centers for Disease

Control and Prevention (CDC) reported three cases of infection with *Salmonella enterica* serovar Tennessee in infants in Canada and the USA. Infection occurred due to contaminated powdered infant formula (CDC, 1993). Also, *Salmonella enterica* serovar Senftenberg isolated from infant formula milk was reported (Rushdy et al., 1998).

Peanut butter, which usually contains over 90% peanuts with optional added sweeteners, salt and stabilizers (Grasso et al., 2010), constitutes the largest segment of peanut consumption. This is due to the increase in the consumption of peanut butter from 1999 to 2008 while peanut candy and snack consumption has not (USDA, 2010). Peanut butter has low a_w (less than 0.35) which precludes the growth of foodborne pathogens (He et al., 2013). But unfortunately, multistate outbreaks caused by peanut butter have been reported. A total of 628 persons from 47 states were infected with *S.* Tennessee traced to contaminated peanut butter in 2007. Symptoms of infection included diarrhea, abdominal cramps, fever and dysuria (CDC, 2007b). Between September 2008 and April 2009, there was a large multistate outbreak caused by *Salmonella* Typhimurium. This outbreak

resulted in 714 illnesses, 166 hospitalizations and 9 deaths. The major source of these *S.* Typhimurium infections was peanut butter (Cavallaro et al., 2011). Also, in 2012, 41 cases of *Salmonella enterica* serovar Bredeney infections were identified in 20 states (CDC, 2013).

Peanut butter is usually pasteurized by conventional heating at temperatures of 70 to 75°C before packaging (Ha et al., 2013). But Ma et al. (2009) reported that thermal treatment at 71°C for 50 min resulted in 2 log reductions of *Salmonella* in peanut butter (a_w = 0.45, pH = 5.12). Shachar and Yaron (2006) also reported that 90°C treatment for 30 min reduced *Salmonella* by about 3 log CFU/g. Conventional heating of peanut butter is a time and energy intensive intervention which consumes large amounts of water. Moreover, food ingredients are heat sensitive which means that food quality deterioration can occur during conventional heating (Chemat et al., 2011). Heating of peanut butter is primarily conductive in nature, and thus much heat has to be applied before the cold spot located at or near the geometric center of product is raised sufficiently to inactivate pathogens, resulting in over-heating of product near the periphery. Especially, peanut

butter has a high oil contents which is susceptible to lipid oxidation resulting in rancidity and off-flavor (Riveros et al., 2010). Due to holding food samples at high temperature for an extended time, lipid oxidation is increased because high temperature decreases the activation energy needed for oxidation (Kanner, 1994). Based on these limitations, there is a need to develop new intervention strategies involving shorter time intervals to reduce *Salmonella* in peanut butter.

Microwave heating has been used for food processing over a period of several decades. Microwave heating can be used in food processing, including drying, thawing, tempering, baking, pasteurization and sterilization of food materials. In contrast to conventional heating, microwave radiation can penetrate the material directly (without any need for an intermediate medium to transfer heat); and also, microwave heating allows for volumetric heating of materials (Zhu et al., 2007). Therefore, microwave treatment can process foods in a shorter time with higher efficiency and with fewer changes in flavor and nutritional qualities compared to conventional heating (Vadivambal and Jayas, 2010). There are

two major frequencies for microwave processing of foods: 2,450 MHz is usually used in domestic microwave ovens and 915 MHz is mainly utilized in industrial microwave equipment (Datta and Davidson, 2000). However, most industrial microwave systems operate at 915 MHz in the USA because they enable greater penetration depth than those of 2,450 MHz (Wang et al., 2003).

There have been many research studies which have confirmed the effect of conventional heat treatment on inactivation of pathogens in peanut butter (He et al., 2011, 2013; Ma et al., 2009; Shachar and Yaron, 2006). However, conventional thermal treatment is not effective for reducing pathogens in peanut butter because of the long treatment time required. Therefore, in this study, I evaluated the effect of a 915 MHz microwave system on the inactivation of *Salmonella* Senftenberg, Typhimurium and Tennessee in creamy peanut butter. Unlike the other two *Salmonella* serovars, there have been no outbreaks traced to *S.* Senftenberg in peanut butter. But *S.* Senftenberg has reportedly been isolated from peanut butter (Burnett et al., 2000); therefore, I also investigated the effect of a 915 MHz microwave

system on the inactivation of *S.* Senftenberg. Also, changes of color, acid, and peroxide values of peanut butter were studied.

II-1.2. Materials and Methods

Bacterial strains and cell suspension *S.* Senftenberg KVCC 0590 and *S.* Tennessee KVCC 0592 were obtained from the Korea Veterinary Culture Collection (KVCC; Anyang, Republic of Korea) and *S.* Typhimurium DT 104 was obtained from the bacteria culture collection of Seoul National University (Seoul, Republic of Korea) for this study. Stock cultures were prepared by mixing 0.7 ml of cultures grown in tryptic soy broth (TSB; Difco, BD, Sparks, MD) for 24 h at 37°C with 0.3 ml of sterile 50% (v/v) glycerol and kept frozen at –80°C. Working cultures were streaked onto tryptic soy agar (TSA; Difco, BD), incubated at 37°C for 24 h and stored at 4 °C. Each strain of *S.* Senftenberg, *S.* Typhimurium, and *S.* Tennessee was cultured in 5 ml TSB at 37°C for 24 h, harvested by centrifugation at 4000 × *g* for 20 min at 4°C, and washed three times with sterile 0.2% peptone water (PW; Bacto, Sparks, MD). The final pellets were resuspended in sterile 0.2% PW, corresponding to approximately 10^8-10^9 CFU/ml.

Sample preparation and inoculation Experiments were performed using commercially processed creamy peanut butter purchased at a local grocery store (Seoul, Republic of Korea) and stored at room temperature (22 ± 1°C). The composition of the peanut butter (in the order listed on the product label) consisted of roasted peanuts, sugar, hydrogenated vegetable oil (cottonseed, soybean and rapeseed oil) and salt. The nutrition facts label indicated 16 g of fat, 7 g of protein, 6 g of total carbohydrate and 150 mg of sodium per each 32 g serving. Thirty g samples of peanut butter were aseptically placed in sterile 100 ml Pyrex glass beakers. For inoculation, 0.3 ml of culture was added to each sample and thoroughly mixed for 2 min with a sterile spoon to ensure even distribution of the pathogen. Uniform distribution of inoculum was confirmed by similar log CFU counts (log 5 – 6 CFU/g) on xylose lysine desoxycholate agar (XLD; Difco) that were obtained from 1 g sub-samples of inoculated peanut butter taken from three randomly selected locations. After inoculation, approximately 5 g was removed to obtain 25 g of inoculated sample. Water activity of inoculated peanut butter was 0.26 and pH was 5.99. A_w and pH were measured by an

Aqualab model 4TE a_w meter (Decagon Devices, Pullman, WA) and a pH meter (Mettler-Toledo, Switzerland).

Microwave heating treatment Microwave treatment was performed in a previously described apparatus (Sung and Kang, 2014). For treatment, 25 g of peanut butter (adjusted to a_w = 0.26 with addition of sterile distilled water) was placed in a 100 ml Pyrex beaker. For the inactivation study, an inoculated sample-filled beaker was placed at the center of the turntable inside the cavity and subjected to microwave heating at 3 different power levels (2, 4, and 6 kW) for up to 5 min. For temperature measurements, the geometric center temperature of a non-inoculated sample in a beaker was measured with a fiber optic sensor (FOT-L; FISO Technologies Inc., Quebec, Canada) connected to a signal conditioner (TMI-4; FISO Technologies Inc., Quebec, Canada). To assess quality changes during microwave heating, non-inoculated sample-filled beakers were treated with microwave heating at 3 different power levels (2, 4, and 6 kW) for 5 min.

Bacterial enumeration After microwave heating treatment, 25 g of sample was mixed with 50 ml of 0.2% PW pre-chilled in ice-water to rapidly cool the sample, thus reducing the effect of residual heat. Then, the sample and 0.2% PW mixture was diluted with 175 ml of sterile 0.2% PW and homogenized for 2 min in a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1 ml aliquots of homogenized samples were ten-fold serially diluted in 9 ml of sterile 0.2% PW, and 0.1 ml of sample or diluent was spread-plated onto XLD for the enumeration of *S.* Senftenberg, Typhimurium, and Tennessee. Where low populations of surviving cells were anticipated, 1 ml aliquots of the original homogenate were equally distributed between four plates and spread-plated. All plates were incubated at 37°C for 24 h and colonies were counted. Experiments were conducted three times.

Acid value, peroxide value and color measurement After 5 min of microwave heating treatment, the acid and peroxide values of peanut butter were measured as indicators of lipid oxidation. Acid value titrations were

determined according to the American Oil Chemists' Society (AOCS) Cd 3d-63 (1998). Peroxide values were determined by iodometric titration according to AOCS Ja 8-87 (2009). Acid value is milligrams of potassium hydroxide necessary to neutralize the free acid in 1 g of sample and peroxide value is the amount of peroxide oxygen per 1 kg of sample. Also, Hunter's color values (*L*, *a* and *b*) were measured using a Minolta colorimeter (model CR300, Minolta Co., Osaka, Japan) after 5 min of microwave heating. Untreated samples were used as controls. *L*, *a,* and *b* values indicate color lightness, redness, and yellowness of the sample, respectively. Experiments were conducted three times.

Statistical analysis All data were analyzed by one-way ANOVA using the Statistical Analysis System (SAS Institute, Cary, NC, USA) and Duncan`s multiple range test to determine if there were significant differences ($P < 0.05$) in mean values. Microbial counts were transformed to $\log_{10}$ values prior to analysis.

II-1.3. Results

Initial populations of *S.* Senftenberg, *S.* Typhimurium, and *S.* Tennessee in inoculated peanut butter were 5.53, 6.27 and 6.00 log CFU/g, respectively. In uninoculated samples, populations of the pathogens were below the detection limit (1.0 log CFU/g). Microwave heating effectively reduced levels of the three *Salmonella* serovars in peanut butter.

The time-temperature profiles of peanut butter during microwave heating at 3 power levels (2, 4 and 6 kW) are shown in Fig. II-1. After 5 min of microwave heating, the center temperatures of peanut butter increased to 22, 49 and 101°C at 2, 4 and 6 kW, respectively. Two kW of microwave heating did not generate heat in peanut butter; however, 6 kW increased the temperature of peanut butter to nearly 100°C. Differences in microwave power greatly affected temperature increase in peanut butter.

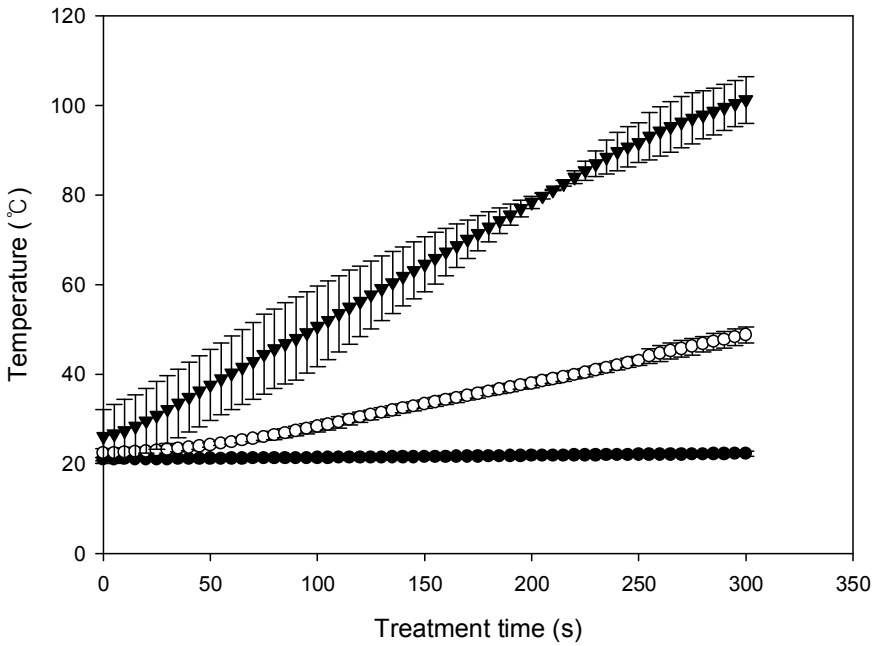


Figure II-1. Time-temperature profiles of the geometric center of peanut butter during microwave heating at 2 kW (●), 4 kW (○) and 6 kW (▼).

Reductions of *Salmonella* in peanut butter are shown in Fig. II-2 to II-4. Fig. II-2 shows the inactivation effect of 3 different microwave power levels (2, 4 and 6 kW) on *S.* Senftenberg in peanut butter. Microwave heating at 2 kW did not significantly reduce *S.* Senftenberg. Microwave heating at 4 kW for 1, 3 and 5 min reduced this pathogen by 0.11, 0.59 and 1.14 log CFU/g, respectively. Six kW microwave heating for 1, 3 and 5 min inactivated this pathogen by 0.41, 1.60, and 3.28 log CFU/g, respectively.

Fig. II-3 shows the inactivation of *S.* Typhimurium in peanut butter by 3 different microwave power settings (2, 4 and 6 kW). The reduction trend of this pathogen was similar to that of *S.* Senftenberg. Two kW microwave heating did not have any effect on microbial inactivation. Four kW microwave heating reduced *S.* Typhimurium by 0.16, 0.44 and 1.18 log CFU/g after 1, 3 and 5 min, respectively. This pathogen was reduced by 0.49, 1.85 and 3.24 log CFU/g when treated with microwave heating at 6 kW for 1, 3 and 5 min, respectively.

Fig. II-4 shows the effect of microwave heating on inactivation of *S.* Tennessee in peanut butter. The reduction trend of *S.* Tennessee in peanut

butter by microwave heating was similar to those of *S.* Senftenberg and *S.* Typhimurium. Microwave heating at 2 kW had no effect on numbers of *S.* Tennessee in peanut butter. Microwave heating at 4 kW for 1, 3 and 5 min reduced this pathogen by 0.11, 0.68 and 1.48 log CFU/g, respectively, and 6 kW microwave heating for 1, 3 and 5 min inactivated this pathogen by 0.94, 2.27 and 4.26 log CFU/g, respectively.

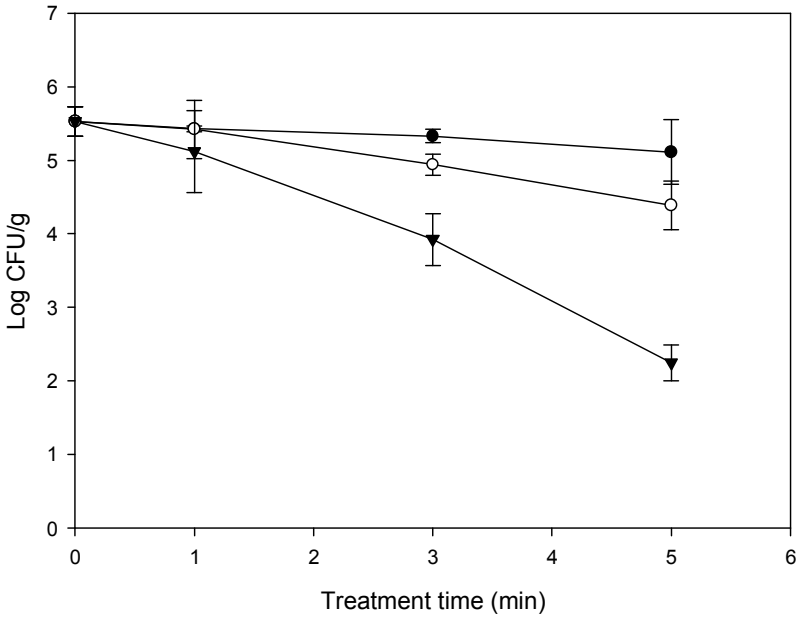


Figure II-2. Survival curves for *S.* Senftenberg KVCC 0590 in peanut butter treated with microwave heating at 2 kW (●), 4 kW (○) and 6 kW (▼).

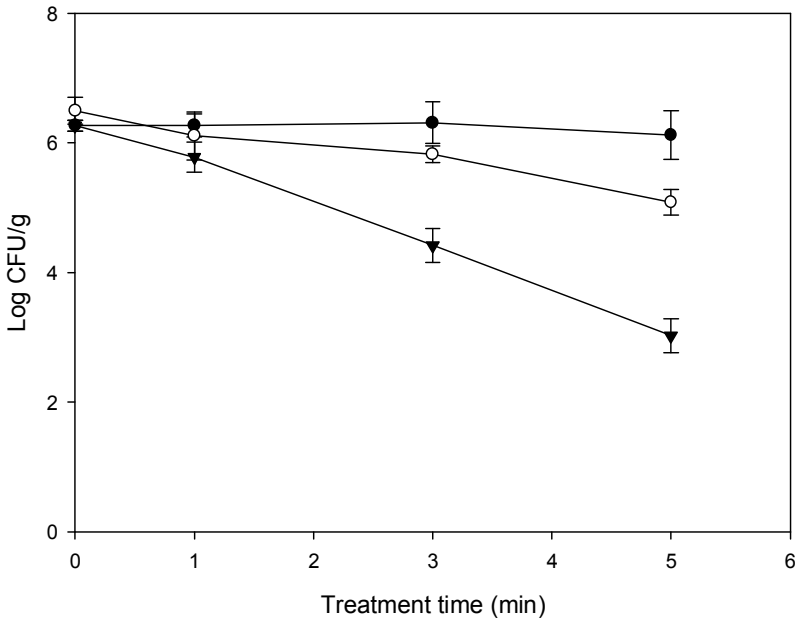


Figure II-3. Survival curves for *S.* Typhimurium DT 104 in peanut butter treated with microwave heating at 2 kW (●), 4 kW (○) and 6 kW (▼).

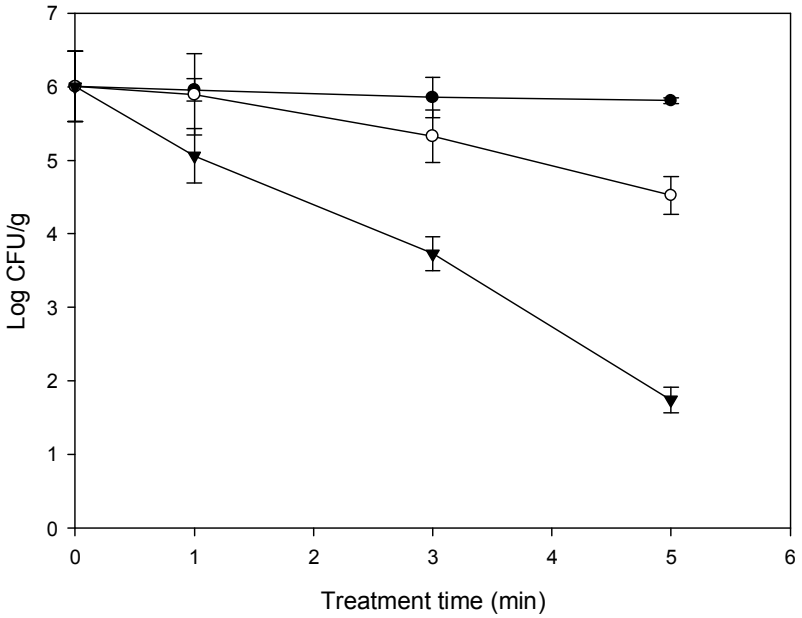


Figure II-4. Survival curves for *S.* Senftenberg KVCC0592 in peanut butter treated with microwave heating at 2 kW (●), 4 kW (○) and 6 kW (▼).

Table II-1 shows acid and peroxide value changes during microwave heating. Acid and peroxide values of microwave heat-treated samples were not significantly different from those of non-treated samples. Both acid and peroxide values slightly increased after microwave heating but these differences were not significant.

Hunter's color values of non-treated and microwave-heated peanut butter are shown in Table II-2. *L, a,* and *b* values of microwave-treated samples were not significantly different from those of non-treated samples. *L* values decreased as microwave power increased from 0 (non-treated sample) to 6 kW, but these differences were not significant.

Table II-1. Acid and peroxide values[a] of 915MHz microwave heat-treated peanut butter.

Microwave power (kW)	Acid value (mg KOH/g)	Peroxide value (mEq/kg)
0 (non-treated)	1.04 ± 0.03 A	10.85 ± 0.76 A
2	1.08 ± 0.04 A	11.07 ± 0.56 A
4	1.09 ± 0.08 A	11.07 ± 0.94 A
6	1.12 ± 0.02 A	11.50 ± 0.78 A

[a] Mean of three replications ± standard deviation. Values followed by the same letters within the column are not significantly different ($P > 0.05$).

Table II-2. Hunter's color *L* (lightness), *a* (redness), and *b* (yellowness) values[a] of 915MHz microwave heat-treated peanut butter.

Treatment	Parameter		
	L	*a*	*b*
Control	60.93 ± 1.37 A	5.25 ± 2.20 A	35.40 ± 2.83 A
2 kW	60.58 ± 1.23 A	5.35 ± 2.36 A	35.46 ± 2.46 A
4 kW	58.19 ± 1.99 A	5.57 ± 2.90 A	35.19 ± 3.23 A
6 kW	57.48 ± 3.15 A	6.09 ± 2.81 A	34.89 ± 3.12 A

[a] Mean of three replications ± standard deviation. Values followed by the same letters within the column are not significantly different ($P > 0.05$).
[b] Color parameters are *L* (lightness), *a* (redness), and *b* (yellowness).

II-1.4. Discussion

A number of studies have reported on the inactivation of foodborne pathogens in peanut butter by conventional heating (He et al., 2013; Ma et al., 2009; Shachar and Yaron, 2006). But, to date, no studies have evaluated the effect of microwave heating on inactivation of *Salmonella* in peanut butter. In the present study, 6 kW and 915 MHz microwave heating for 5 min reduced *Salmonella* by 3.24 to 4.26 log CFU/g.

Microwaves are part of the electromagnetic spectrum and fall within a frequency range of 300 MHz to 300 GHz. The most important mechanism of microwave heating is its interaction with water molecules which can generate heat inside of foods (Papadopoulou et al., 1995), whereas conventional heating depends on conduction and convection to transport heat from the heating source of intermediate medium (e.g., water or steam) to the interior of the product, which requires a relatively longer period of time (Mullin, 1995). There are two major frequencies used for microwave heating, 915 and 2,450 MHz. These two frequencies were approved for

heating applications in North America by the FCC (Federal Communications Commission) (Ramaswamy and Tang, 2008) and produce wavelengths of 0.33 and 0.12 m in free space, respectively (Tanaka et al., 1999). The penetration depth of a wave is proportional to its wavelength, thus, a 915 MHz microwave shows deeper penetration depth than a 2,450 MHz microwave. Because of the longer penetration depth, I used 915 MHz microwave heating for inactivation of *Salmonella* in peanut butter.

Pasteurization of peanut butter by conventional heating has been investigated many times (He et al., 2013; Ma et al., 2009; Shachar and Yaron, 2006), but this method has not been effective for reducing *Salmonella* in peanut butter. Because of the ineffectiveness of conventional heating, many researchers have focused on non-thermal inactivation methods to control *Salmonella* in peanut butter. Hvizdzak et al. (2010) used electron beam treatment for reduction of *Salmonella* in peanut butter. Three kGy of electron beam radiation reduced *S.* Tennessee and *S.* Typhimurium by 6.75 and 4.86 log CFU/g, respectively, when plated on a selective medium (XLD) for enumeration. Ban and Kang (2014) reported that 3 kGy

of gamma irradiation reduced *S.* Typhimurium in three types of peanut butter by 3.5 to 4.0 log CFU/g. D'Souza et al. (2012) reported that high hydrostatic pressure under various conditions (400 to 600 MPa and 4 to 18 min) reduced cell numbers of a *Salmonella enterica* serovar cocktail in creamy peanut butter by 1.6 to 1.9 log CFU/g. Ha et al. (2013b) used radio frequency for inactivation of *S.* Typhimurium and *E. coli* O157:H7 in peanut butter cracker sandwiches. Radio frequency treatment for 90 s reduced these pathogens by 4.55 and 5.32 log CFU/g, respectively. However, these control methods are not suitable for industry. During electron beam and gamma irradiation treatment, peanut butter was spread into a thin layer to enhance irradiation absorption. High hydrostatic pressure treatment reduced *Salmonella* by less than 2.0 log CFU/g which is insufficient to control large populations of *Salmonella* and radio frequency treatment involved peanut butter cracker sandwiches, not just peanut butter. In my study, using a 25 g sample of peanut butter and utilizing a 915 MHz microwave, I effectively reduced *S.* Senftenberg, Typhimurium and Tennessee by 3.24 to 4.26 log CFU/g within 5 min. Microwave heating is thus more suitable for

pasteurization of peanut butter from an industrial standpoint.

Acid and peroxide values are indicators of lipid oxidation of foods. Lipid oxidation is a major cause of quality deterioration of foods containing high levels of lipids and results from exposure to high temperature. Gao et al. (2011) reported that peroxide values of almonds increased after radio frequency heating. But in this study, acid and peroxide values were not affected by microwave heating. Lipid oxidation can be induced by some non-thermal treatments. Zheng et al. (2010) reported that pulsed electric field-treated peanut oil showed higher peroxide values than that of the control. Another study confirming that peanut butter treated by electron beams showed increased peroxide values was also reported (El-Rawas et al., 2012).

The first impression a consumer has of a given food product is the appearance of the product. Color, a part of the appearance, should be within an acceptable range. If the color is unacceptable, other quality factors, such as flavor and texture, are not likely to be judged at all (Francis, 1995). Ha et al. (2013b) reported that there were no significant color changes during

pasteurization of peanut butter crackers by radio frequency treatment. But color changes in peanut butter resulting from electron beam and gamma irradiation were reported. Ban and Kang (2014) reported that gamma irradiation in excess of 3 kGy decreased the L^* value of peanut butter. And El-Rawas et al. (2012) reported that during electron beam radiation color changes of peanut butter occurred. In this study, microwave heating did not affect the color values of peanut butter.

In conclusion, this study evaluated the feasibility of 915 MHz microwave heating as a novel pasteurization technology for peanut butter. Treatment with a 915 MHz microwave at 6 kW effectively reduced *Salmonella* in peanut butter and did not affect acid value, peroxide value, color, nor moisture content (data not shown) of peanut butter. Nine hundred fifteen MHz microwave heating could be utilized by the peanut butter industry. However, further investigations need to be performed to confirm which factors can affect microwave heating of peanut butter, such as frequency and a_w.

II-2. Influence of water activity on inactivation of foodborne pathogens in peanut butter by 915 MHz microwave heating

II-2.1. Introduction

Escherichia coli O157:H7 is an increasingly common cause of illness, including bloody diarrhea and hemolytic uremic syndrome (Besser et al., 1999). *Salmonella enterica* serovar Typhimurium is the most commonly isolated *Salmonella* serotype and causes non-typhoidal salmonellosis which has a symptom of self-limiting gastroenteritis (Boyle et al., 2007). *Listeria monocytogenes* is a one of the most widespread gram-positive bacteria which causes abortion, stillbirth, neonatal sepsis, meningitis, sepsis, and gastroenteritis (Salamina et al., 1996). Robertson et al. (2016) reported that there were 163 outbreaks from 2007 to 2012, in the United States, comprising 89 *E. coli*, 56 *Salmonella*, and 11 *L. monocytogenes* cases. The 4,132 cases of illnesses were reported due to 163 outbreaks, *Salmonella* comprised 68%, STEC comprised 26% of illnesses, and *L. monocytogenes* showed the highest fatality (38%). Low a_w foods have usually been considered safe regarding foodborne pathogens because the optimum a_w for growth of these pathogens is over 0.95. But, unfortunately, there have been

several outbreaks due to low a_w foods contaminated with *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes*. In 1998, there was an outbreak caused by *E. coli* O157:H7 in southern Ontario, Canada. The major source of contamination was dry fermented Genoa salami (Williams et al., 2000). There was a large outbreak in Norway and Finland due to *S.* Typhimurium-contaminated chocolate in 1987. Because of this outbreak 361 people were infected with *S.* Typhimurium and many young children developed acute hemorrhagic diarrhea (Kapperud et al., 1990). Also, a *L. monocytogenes* infection outbreak in Finland was traced to contaminated butter (Lyytikäinen et al., 2000).

Peanut butter has low a_w which disrupts the growth of foodborne pathogens, and generally, is pasteurized by conventional heating at 70 to 75°C (Ha et al., 2013b). But, unfortunately, multistate outbreaks caused by peanut butter have been reported. In 2007 in the USA, there was a large outbreak of salmonellosis due to consumption of *Salmonella enterica* serovar Tennessee-contaminated peanut butter. Because of this outbreak, 425 people were infected with *S.* Tennessee and 71 people were hospitalized

(CDC, 2007a). In 2008 – 2009, a multistate outbreak of *S.* Typhimurium infections linked to peanut butter occurred. This outbreak affected 714 people from 46 states of the USA; 24% of whom required hospitalization and resulted in 9 deaths (CDC, 2010a). Also, in 2012 in the USA, 42 cases of *Salmonella* Bredeney infections linked to peanut butter consumption were reported (CDC, 2012). And also, there have been several studies which confirmed that conventional heating is not sufficient to inactivate *Salmonella* in peanut butter. Ma et al. (2009) reported that conventional thermal treatment at 71°C for 50 min reduced *Salmonella* Tennessee by just 2 log CFU/g. He et al. (2011) also reported that thermal treatment at 72°C for 60 min reduced *Salmonella enterica* by less than 2 log CFU/g in peanut butter with 0.4 a_w.

Microwave heating is a form of dielectric heating which is used industrially for the processing of food and also used domestically for cooking or thawing of food. Microwave irradiation produces efficient volumetric heating by utilizing the ability of microwave which can penetrate the material directly without any need of intermediate heat transfer medium

(Zhu et al., 2007). Microwave heating is greatly affected by water in food because of the dipolar nature of water. When an electric field is applied to water, the dipolar water molecules try to realign in the direction of the electric field. This million times per second realignment due to the high frequency of microwaves cause internal friction of water molecules resulting in the volumetric heating of food. Because of this reason, microbial inactivation of food by microwave heating is focused on foods which have moisture contents higher than 50%, such as, milk (Choi et al., 1993), juice (Cañumir et al., 2002), meat (Shamis et al., 2008) and poultry (Pucciarelli and Benassi, 2005).

Water is one of the key factors which control the effect of microwave heating. And also, the a_w of food affects the heat resistance of microorganisms in food. Goepfert et al. (1970) reported that reduced a_w increased the D-value of salmonellae. But there has been no study investigating the effect of a_w of foods on microwave heating and inactivation of pathogens in foods by microwave heating treatment. Recently, Song and Kang (2016a) reported that microwave heating is

effective for the pasteurization of peanut butter. Therefore, in this study I evaluated the effect of a_w on inactivation of three foodborne pathogens (*E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*) in peanut butter by microwave heating and obtained the inactivation kinetics of the three pathogens.

II-2.2. Materials and Methods

Bacterial strains and cell suspension Strains of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 19115, DT 104), and *L. monocytogenes* (ATCC 15313, ATCC 19111, ATCC 19115) were obtained from the bacteria culture collection of Seoul National University (Seoul, Republic of Korea) for this study. Stock cultures were kept frozen at –80°C in 0.7 ml of Tryptic Soy Broth (TSB; Difco, BD, Sparks, MD) and 0.3 ml of sterile 50% (V/V) glycerol. Working cultures were streaked onto Tryptic Soy Agar (TSA; Difco, BD), incubated at 37°C for 24 h and stored at 4°C. Each strain of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was cultured in 5 ml TSB at 37°C for 24 h, harvested by centrifugation at 4,000 × *g* for 20 min at 4°C and washed three times with sterile distilled water. The final pellets were resuspended in sterile distilled water, corresponding to approximately 10^8-10^9 CFU/ml. Mixed culture cocktails were prepared by blending together equal volumes of each test strain.

Sample preparation and inoculation The peanut butter used for this study was purchased at a local grocery store (Seoul, Republic of Korea) and stored at room temperature (22 ± 1˚C). Twenty-five g of peanut butter samples were aseptically placed in sterile 100 ml Pyrex beakers. For inoculation, 0.2 ml of culture was inoculated into the sample and thoroughly mixed for 1 min with a sterile spoon to ensure even distribution of the pathogens. A_w of inoculated peanut butter was 0.30. To increase peanut butter a_w, a select volume of sterile distilled water was mixed into the inoculated peanut butter samples to adjust the a_w to 0.4 and 0.5. Generally peanut butter has water activity range from 0.20 to 0.33 (Burnett et al., 2000). But some studies used peanut butter with a_w 0.4 to 0.5 (Ha et al., 2013b; Ma et al., 2009; Shachar and Yaron, 2009) so I used these a_w (0.3, 0.4, and 0.5). Uniform distribution of inoculum was confirmed by similar log CFU counts (log 5 – 6 CFU/g) on Sorbitol MacConkey agar (SMAC; Difco), Xylose Lysine Desoxycholate agar (XLD; Difco) and Oxford Agar Base with antimicrobial supplement (OAB; MB Cell) for enumeration of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes*, respectively, that

were obtained from 1 g sub-samples of inoculated peanut butter taken from three randomly selected locations. I removed excess peanut butter to obtain 25 g of inoculated sample because total sample weight was increased due to inoculation and adjustment of a_w. Water activity of inoculated peanut butter was measured with an Aqualab model 4TE a_w meter (Decagon Devices, Pullman, WA).

Microwave heating treatment Microwave treatment was performed in a previously described apparatus (Sung and Kang, 2014). The beaker containing 25 g of peanut butter sample was located at the center of the turntable. For temperature measurements, the geometric center temperature of a non-inoculated sample adjusted to 0.3, 0.4, and 0.5 a_w in a beaker was measured by a fiber optic sensor (FOT-L; FISO Technologies Inc., Quebec, Canada) connected to a signal conditioner (TMI-4; FISO Technologies Inc., Quebec, Canada). For the inactivation study, 25 g of peanut butter with different a_w was placed in a 100 ml Pyrex beaker. An inoculated sample-filled beaker was subjected to microwave heating at 3 different power levels

(2, 4, and 6 kW) for up to 5 min.

Bacterial enumeration After microwave heating treatment, 25 g of sample was mixed with 25 ml of 0.2% peptone water (PW). Then, the sample and 0.2% PW mixture was diluted in 200 ml of sterile 0.2% PW and homogenized for 2 min in a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1 ml aliquots of homogenized samples were 10-fold serially diluted in 9 ml of sterile 0.2% PW, and 0.1 ml of sample or diluent was spread-plated onto SMAC, XLD, and OAB for enumeration of the three pathogens. Where low populations of surviving cells were anticipated, 1 ml aliquots of the original homogenate were equally distributed between four plates and spread-plated. All plates were incubated at 37°C for 24 h and colonies were counted.

Modeling of survival curves and t_d and t_{5d} values calculation Survival curves for microwave heating treatments were obtained by plotting the logarithm of the surviving population versus treatment time (min). To fit

survival curves and calculate parameters for fitting the model, the Geeraerd and Van Impe Inactivation model Fitting Tool (GInaFiT) was used (Geeraerd et al., 2005). Because my survival curves were obtained under non-isothermal conditions and showed a shouldering effect, the curves were fitted with the Weibull model (equation 1) (Mafart et al., 2002) and Log-Linear + Shoulder model (equation 2) (Geeraerd et al., 2000).

$$\log\left(\frac{N}{N_0}\right) = -\left(\frac{t}{\delta}\right)^p \quad (1)$$

where N = the number of survivors at microwave heating time, N_0 = initial population of pathogen, t = treatment time (min), δ = scale parameter (min), and p = shape parameter.

$$\log\left(\frac{N}{N_0}\right) = -\frac{k_{max}*t}{\ln(10)} + \log\left(\frac{Exp(k_{max}*Sl)}{1+(Exp(k_{max}*Sl)-1)*Exp(-k_{max}*t)}\right) \quad (2)$$

where N = the number of survivors at microwave heating time, N_0 = initial population of pathogen, t = treatment time (min), k_{max} = maximum inactivation rate (1/min), and Sl = shoulder length.

The goodness of fit of the two models was evaluated by the coefficient of determination (R^2) and mean squared error (MSE). The R^2 value is the coefficient of determination and a larger R^2 value (as it approaches 1)

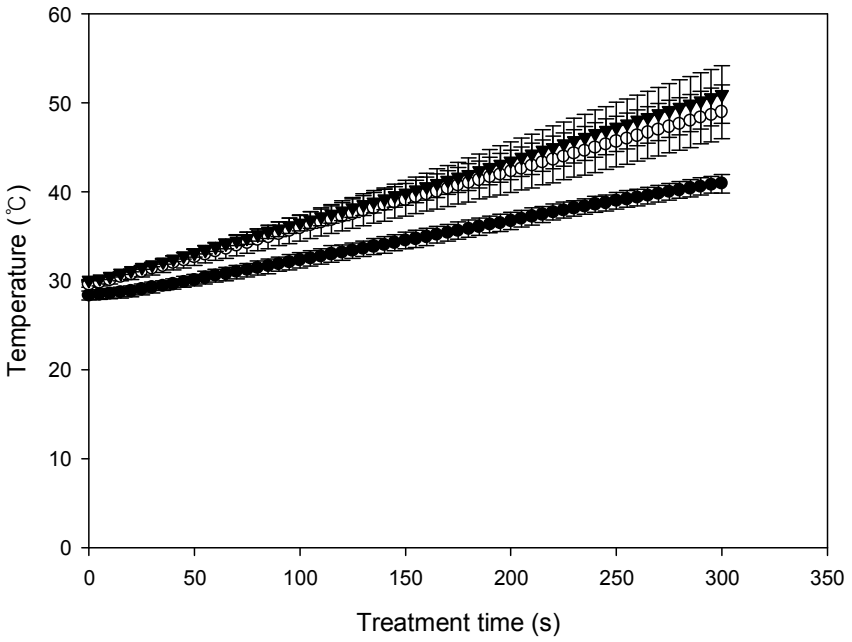
indicates better the fit of the model to the data. MSE is the average of the squares of the error and a smaller MSE value (as it approaches 0) indicates better fit of the model to the data. And also, t_d and t_{5d} values were calculated based on Weibull (equation 1) and Log-Linear + Shoulder model (equation 2) by using Microsoft Excel 2010 software package (Microsoft Coporation).

Statistical analysis All data were analyzed with one-way ANOVA using Statistical Analysis System (SAS Institute, Cary, NC, USA) and Duncan`s multiple range test to determine if there were significant differences ($P < 0.05$) in mean values of microorganism populations, t_d and t_{5d} values. Microbial counts were transformed to log values for analysis. One log was used for calculations in the case of populations below the detection limit.

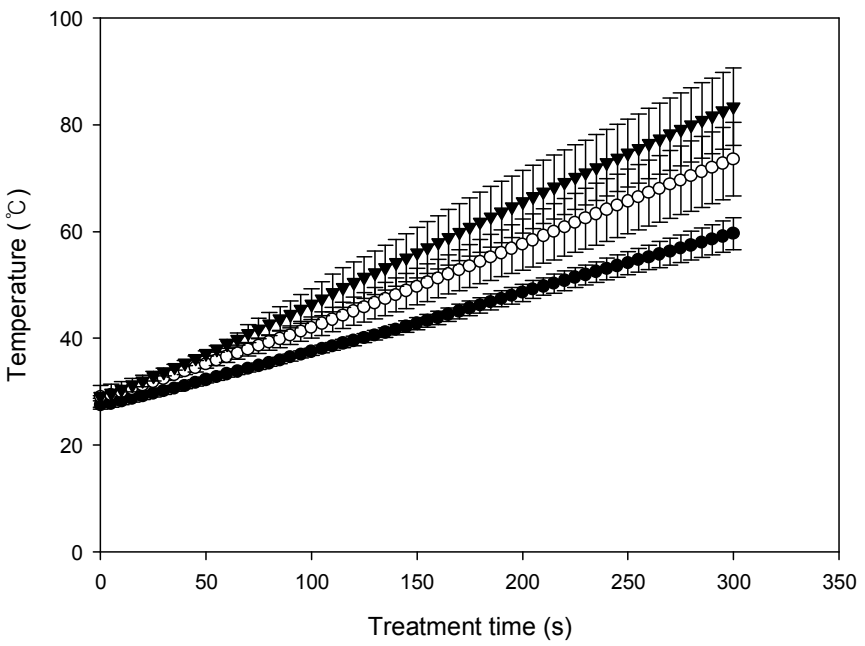
II-2.3. Results

Average center temperatures of peanut butter with different a_w are shown in Fig. II-5. Differences in temperature between peanut butters of different a_w were detected during 1 to 5 min of 915 MHz microwave treatment. After 5 min of 6 kW microwave treatment, there was ca. 18°C difference between 0.3 a_w (94.76°C) and 0.4 a_w peanut butter (111.8°C) and ca. 15°C between 0.4 a_w (111.8°C) and 0.5 a_w peanut butter (127.35°C). Following microwave treatment for 5 min at 4 kW, those temperature differences were 14°C between 0.3 (59.64°C) and 0.4 a_w (73.52°C) peanut butter and 10°C between 0.4 (73.52°C) and 0.5 a_w (83.38°C) peanut butter. Two kW microwave treatment for 5 min increased the temperature of peanut butter (0.3, 0.4 and 0.5 a_w) by up to 40.92, 48.97 and 50.93°C, respectively.

(a)


Temperature (℃)
Treatment time (s)
0
10
20
30
40
50
60
0
50
100
150
200
250
300
350

(b)


Temperature (℃)
Treatment time (s)
0
20
40
60
80
100
0
50
100
150
200
250
300
350

(c)

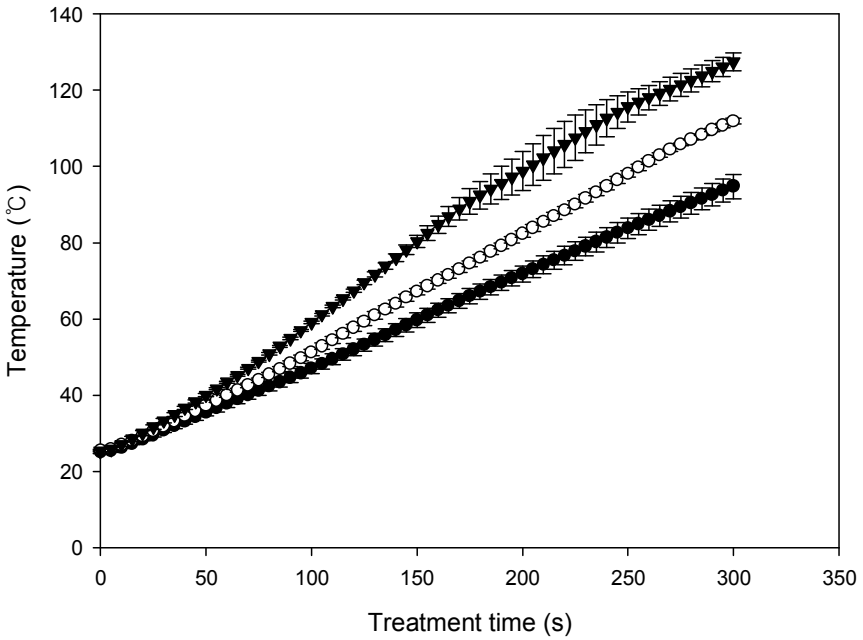


Figure II-5. Temperature histories of peanut butter of different a_w treated with different microwave power levels. (a) 2 kW, (b) 4 kW, (c) 6 kW. ● 0.30 a_w; ○ 0.40 a_w; ▼ 0.50 a_w.

Reductions in the viable counts of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* cells in peanut butter with different a_w during microwave heating at different power levels are shown in Table II-3 to II-5. No significant ($P > 0.05$) log reductions of the three pathogens in any of the peanut butter samples were observed after 2 kW microwave heating for 5 min. *E. coli* O157:H7 survival curves following microwave heating at different power levels of peanut butter with different a_w are shown in Table II-3. Treatment at 4 kW for 5 min inactivated populations of this pathogen in peanut butter (0.3, 0.4, and 0.5 a_w) by about 1.26, 1.29 and 1.24 log CFU/g, respectively. The 6 kW treatment for 5 min reduced *E. coli* O157:H7 in peanut butter (0.3, 0.4, and 0.5 a_w) by 2.78, 3.03 and 4.03 log CFU/g, respectively.

Table II-4 shows survival curves of *S.* Typhimurium in peanut butter with different a_w after microwave heating. Microwave heating at 4 kW for 5 min reduced *S.* Typhimurium in peanut butter (0.3, 0.4 and 0.5 a_w) by 1.18, 1.67 and 1.98 log CFU/g, respectively. Six kW microwave heating for 5 min inactivated this pathogen by 2.50, 3.51 and > 5.17 log CFU/g (0.3, 0.4 and

0.5 a_w peanut butter), respectively.

Reduction of *L. monocytogenes* in peanut butter of varying a_w by different microwave heating power levels is shown in Table II-5. Inactivation of *L. monocytogenes* shows a similar trend to that of *S.* Typhimurium. Treatment at 4 and 6 kW for 5 min inactivated this pathogen by 0.41 and 1.97 (0.3 a_w), 1.13 and 2.80 (0.4 a_w) and 1.25 and 3.82 log CFU/g (0.5 a_w), respectively. Generally, *L. monocytogenes* showed more resistance to microwave heating than *E. coli* O157:H7 and *S.* Typhimurium and *S.* Typhimurium showed the least resistance. Especially, *S.* Typhimurium in 0.5 a_w peanut butter was reduced to under the detection limit (1.0 log CFU/g, more than 5 log reduction) when treated with 6 kW microwave heating for 5 min, but *L. monocytogenes* was reduced by 3.82 log CFU/g.

Table II-3. Effect of microwave heating at different power levels on the inactivation of *E. coli* O157:H7 in peanut butter with different a_w.

Power (kW)	A_w	Microbial populations of *E. coli* O157:H7 (log CFU/g) [a]					
		Control (0 min)	1 min	2 min	3 min	4 min	5 min
2	0.3	6.67 ± 0.30[a] Aa	6.66 ± 0.05 Aa	6.53 ± 0.21 Aa	6.72 ± 0.21 Aa	6.70 ± 0.11 Aa	6.43 ± 0.22 Aa
	0.4	6.61 ± 0.12 Aa	6.54 ± 0.21 Aa	6.63 ± 0.06 Aa	6.62 ± 0.04 Aa	6.30 ± 0.26 Ab	6.31 ± 0.30 Aa
	0.5	6.59 ± 0.30 Aa	6.55 ± 0.24 Aa	6.78 ± 0.16 Aa	6.65 ± 0.11 Aa	6.48 ± 0.00 Aab	6.67 ± 0.09 Aa
4	0.3	6.76 ± 0.14 Aa	6.61 ± 0.13 Aa	6.50 ± 0.21 Aa	6.46 ± 0.15 Aa	5.90 ± 0.16 Ba	5.50 ± 0.22 Ca
	0.4	6.49 ± 0.16 Aab	6.64 ± 0.08 Aa	6.50 ± 0.10 Aa	6.15 ± 0.16 Ba	5.70 ± 0.09 Cab	5.20 ± 0.27 Da
	0.5	6.25 ± 0.19 Ab	6.42 ± 0.37 Aa	6.36 ± 0.26 Aa	6.06 ± 0.44 Aa	5.48 ± 0.15 Bb	5.01 ± 0.36 Ba
6	0.3	6.80 ± 0.14 Aa	6.45 ± 0.13 ABa	5.97 ± 0.34 Ba	5.30 ± 0.29 Ca	4.54 ± 0.28 Da	4.03 ± 0.39 Ea
	0.4	6.41 ± 0.09 Ab	6.33 ± 0.07 ABa	5.87 ± 0.19 Ba	5.17 ± 0.36 Ca	4.25 ± 0.35 Dab	3.38 ± 0.38 Eab
	0.5	6.69 ± 0.14 Aa	6.56 ± 0.25 Aa	5.76 ± 0.48 Ba	4.80 ± 0.47 Ca	3.93 ± 0.24 Db	2.66 ± 0.32 Eb

[a] Mean of three replications ± standard deviation. Values followed by the same uppercase letters within a row and by the same lowercase letters within a column are not significantly different ($P > 0.05$).

Table II-4. Effect of microwave heating at different power levels on the inactivation of *S.* Typhimurium in peanut butter with different a_w.

Power (kW)	A_w	Microbial populations of *S.* Typhimurium (log CFU/g) [a]					
		Control (0 min)	1 min	2 min	3 min	4 min	5 min
2	0.3	6.31 ± 0.29[a] Aa	6.37 ± 0.09 Aa	6.22 ± 0.45 Aa	6.32 ± 0.25 Aa	6.38 ± 0.24 Aa	6.19 ± 0.19 Aa
	0.4	6.33 ± 0.02 Aa	6.28 ± 0.25 Aa	6.37 ± 0.18 Aa	6.33 ± 0.13 Aa	6.22 ± 0.40 Aa	5.98 ± 0.50 Aa
	0.5	6.16 ± 0.12 Aa	6.32 ± 0.21 Aa	6.15 ± 0.41 Aa	6.24 ± 0.25 Aa	6.10 ± 0.35 Aa	5.94 ± 0.41 Aa
4	0.3	6.24 ± 0.15 Aa	6.14 ± 0.13 Aa	6.07 ± 0.25 Aa	6.03 ± 0.21 Aa	5.46 ± 0.22 Ba	5.02 ± 0.24 Ca
	0.4	6.16 ± 0.15 Aa	6.15 ± 0.15 Aa	5.83 ± 0.30 ABa	5.73 ± 0.12 Bab	5.23 ± 0.22 Cab	4.49 ± 0.11 Db
	0.5	5.91 ± 0.19 ABa	6.10 ± 0.18 Aa	5.67 ± 0.23 ABa	5.47 ± 0.33 Bb	4.82 ± 0.36 Cb	3.93 ± 0.33 Dc
6	0.3	6.30 ± 0.18 Aa	6.03 ± 0.28 Aa	5.54 ± 0.26 Ba	5.30 ± 0.45 Ba	4.23 ± 0.17 Ca	3.80 ± 0.34 Ca
	0.4	5.98 ± 0.03 Ab	5.78 ± 0.24 ABa	5.37 ± 0.14 Ba	4.59 ± 0.33 Cab	3.35 ± 0.41 Db	2.47 ± 0.30 Eb
	0.5	6.17 ± 0.13 Aab	5.79 ± 0.10 Aa	5.16 ± 0.26 Ba	4.09 ± 0.50 Cb	2.73 ± 0.37 Db	> 1.00 ± 0.00 Ec

[a] Mean of three replications ± standard deviation. Values followed by the same uppercase letters within a row and by the same lowercase letters within a column are not significantly different ($P > 0.05$).

Table II-5. Effect of microwave heating at different power levels on the inactivation of *L. monocytogenes* in peanut butter with different a_w.

Power (kW)	A_w	Microbial populations of *L. monocytogenes* (log CFU/g) [a]					
		Control (0 min)	1 min	2 min	3 min	4 min	5 min
2	0.3	4.82 ± 0.50[a] Aa	4.83 ± 0.43 Aa	4.80 ± 0.28 Aa	4.90 ± 0.41 Aa	4.75 ± 0.26 Aa	4.76 ± 0.19 Aa
	0.4	4.89 ± 0.37 Aa	4.75 ± 0.39 Aa	4.83 ± 0.46 Aa	4.75 ± 0.39 Aa	4.69 ± 0.32 Aa	4.68 ± 0.41 Aa
	0.5	5.07 ± 0.12 Aa	4.79 ± 0.43 Aa	4.86 ± 0.49 Aa	4.75 ± 0.35 Aa	4.75 ± 0.45 Aa	4.76 ± 0.33 Aa
4	0.3	5.21 ± 0.33 Aa	5.24 ± 0.23 Aa	5.19 ± 0.16 Aa	5.05 ± 0.27 Aa	4.98 ± 0.32 Aa	4.79 ± 0.36 Aa
	0.4	5.30 ± 0.00 Aa	5.20 ± 0.17 ABa	5.00 ± 0.15 BCa	4.92 ± 0.17 BCa	4.84 ± 0.18 Ca	4.17 ± 0.20 Db
	0.5	5.13 ± 0.16 Aa	5.06 ± 0.10 Aa	5.08 ± 0.04 Aa	4.88 ± 0.16 Aa	4.34 ± 0.17 Bb	3.88 ± 0.22 Cb
6	0.3	5.59 ± 0.26 Aa	5.49 ± 0.14 ABa	5.11 ± 0.22 BCa	4.70 ± 0.41 CDa	4.41 ± 0.24 Da	3.62 ± 0.16 Ea
	0.4	5.52 ± 0.35 Aa	5.33 ± 0.25 Aa	5.16 ± 0.12 Aa	4.43 ± 0.23 Ba	3.56 ± 0.08 Cb	2.72 ± 0.31 Db
	0.5	5.42 ± 0.17 Aa	5.13 ± 0.27 ABa	4.92 ± 0.28 Ba	4.17 ± 0.24 Ca	3.32 ± 0.25 Db	1.60 ± 0.30 Ec

[a] Mean of three replications ± standard deviation. Values followed by the same uppercase letters within a row and by the same lowercase letters within a column are not significantly different ($P > 0.05$).

In this study, I used the Weibull (equation 1) and Log-Linear + Shoulder (equation 2) models to describe the experimental data obtained by microwave heating. Table II-6 shows R^2 and MSE values of these two models to compare their goodness of fit. The R^2 values with the Weibull model were higher than 0.95, except for 5 out of a total of 18 cases. A similar situation was observed in R^2 values with the Log-Linear + Shoulder model which were higher than 0.95, except in 4 cases. The mean R^2 values of each of the two models were 0.945 (Weibull model) and 0.951 (Log-Linear –Shoulder model). Similar trends were observed in MSE values. The MSE values with the Weibull and Log-Linear + Shoulder models were smaller than 0.1 except in 3 cases (Weibull model) and 2 cases (Log-Linear + Shoulder model). The mean MSE values of each of the two models were 0.044 (Weibull model) and 0.045 (Log-Linear + Shoulder model). Instead, I used Weibull and Log-Linear + Shoulder models to calculate t_d and t_{5d} values of pathogens in peanut butter. The Weibull and Log-Linear + Shoulder model parameters are shown in Table II-7.

Table II-6. Comparison of goodness of fit of the Weibull and Log-Linear + Shoulder models for the inactivation of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in peanut butter treated with microwave heating at different power levels.

Pathogens	Power (kW)	A_w	Weibull model		Log linear + Shoulder model	
			R^{2a}	MSE[b]	R^2	MSE
Escherichia coli O157:H7	4	0.3	0.962	0.0152	0.966	0.0137
		0.4	0.954	0.0237	0.956	0.0228
		0.5	0.842	0.0799	0.855	0.0752
	6	0.3	0.981	0.0358	0.982	0.0324
		0.4	0.994	0.0167	0.997	0.0081
		0.5	0.987	0.0594	0.987	0.0574
Salmonella Typhmiurium	4	0.3	0.949	0.0232	0.955	0.0200
		0.4	0.980	0.0136	0.974	0.0173
		0.5	0.959	0.0485	0.969	0.0324
	6	0.3	0.915	0.1243	0.914	0.1256
		0.4	0.976	0.1044	0.979	0.0904
		0.5	0.984	0.1102	0.981	0.1291
Listeria monocytogenes	4	0.3	0.679	0.0215	0.752	0.0151
		0.4	0.937	0.0179	0.934	0.0189
		0.5	0.975	0.0115	0.982	0.0082
	6	0.3	0.977	0.0209	0.969	0.0288
		0.4	0.977	0.0282	0.989	0.0203
		0.5	0.988	0.0407	0.975	0.0894

[a] R^2, regression coefficient.

[b] MSE, mean square error.

Table II-7. Kinetic parameters of the Weibull and Log-Linear + Shoulder models for *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in peanut butter of different a_w treated with microwave heating at 4 and 6 kW.

Pathogens	Power (kW)	A_w	Weibull model		Log linear + Shoulder model	
			δ (min)	p	Sl (min)	k_{max} (1/min)
Escherichia coli O157:H7	4	0.3	4.59 ± 0.18	2.40 ± 0.52	2.80 ± 0.42	1.27 ± 0.25
		0.4	4.32 ± 0.31	2.36 ± 0.31	2.63 ± 0.28	1.37 ± 0.20
		0.5	4.32 ± 0.73	2.83 ± 0.98	2.80 ± 0.95	1.56 ± 0.33
	6	0.3	2.17 ± 0.54	1.27 ± 0.27	0.72 ± 0.67	1.55 ± 0.32
		0.4	2.62 ± 0.46	1.77 ± 0.25	1.64 ± 0.41	2.09 ± 0.23
		0.5	2.04 ± 0.72	1.70 ± 0.73	1.30 ± 0.88	2.61 ± 0.68
Salmonella Typhmiurium	4	0.3	4.75 ± 0.21	2.86 ± 0.76	3.12 ± 0.27	1.49 ± 0.53
		0.4	4.09 ± 0.25	2.58 ± 0.87	2.61 ± 0.58	1.60 ± 0.45
		0.5	3.70 ± 0.57	2.58 ± 0.31	2.59 ± 0.45	1.90 ± 0.06
	6	0.3	2.56 ± 0.43	1.31 ± 0.05	0.83 ± 0.19	1.38 ± 0.41
		0.4	2.39 ± 0.46	1.79 ± 0.56	1.59 ± 0.75	2.47 ± 0.76
		0.5	2.02 ± 0.39	1.83 ± 0.40	1.60 ± 0.65	3.39 ± 0.58
Listeria monocytogenes	4	0.3	17.28 ± 15.04	1.10 ± 0.34	2.45 ± 2.97	0.87 ± 0.71
		0.4	5.03 ± 0.22	3.34 ± 2.10	2.95 ± 1.57	1.50 ± 0.99
		0.5	4.55 ± 0.21	2.72 ± 0.68	2.95 ± 0.41	1.45 ± 0.35
	6	0.3	3.34 ± 0.83	1.92 ± 0.86	2.41 ± 0.10	1.65 ± 0.21
		0.4	2.90 ± 0.53	1.93 ± 0.29	1.89 ± 0.47	2.05 ± 0.10
		0.5	2.97 ± 0.25	2.50 ± 0.34	2.28 ± 0.21	2.94 ± 0.33

Calculated t_d and t_{5d} values are shown in Table II-8. t_d and t_{5d} values based on the Log-Linear Regression model were not computed because this model could not account for the reduction curves obtained by microwave heating. t_d values calculated based on the Weibull model showed no significant differences between different a_w except for 4 kW microwave heating of *S.* Typhimurium. *S.* Typhimurium in 0.5 a_w peanut butter showed a smaller t_d value than that of this pathogen in 0.3 a_w peanut butter. But significant differences in t_{5d} values computed based on the Weibull model were observed. Especially, t_{5d} values of the three pathogens treated with 6 kW microwave heating showed significant differences in all cases. t_{5d} values of the three pathogens in 0.5 a_w peanut butter were significantly lower than those of the three pathogens in 0.3 a_w peanut butter. And *L. monocytogenes* showed reduced t_{5d} values with increasing a_w when treated with 4 kW microwave heating. t_d values of *E. coli* O157:H7, which were calculated based on the Log-Linear + Shoulder model, were not affected by a_w (no significant differences). *S.* Typhimurium in 0.4 and 0.5 a_w peanut butter showed smaller t_d values than those of *S.* Typhimurium in 0.3 a_w peanut

butter and *L. monocytogenes* showed decreased t_d values as a_w increased. t_{5d} values of the three pathogens in 0.5 a_w peanut butter were smaller than those of the three pathogens in 0.3 a_w peanut butter except for *E. coli* O157:H7 and *L. monocytogenes* when treated with 4 kW microwave heating.

Table II-8. Calculated t_d and t_{5d} values for *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in peanut butter with different a_w based on the Weibull and Log-Linear + Shoulder models.

Pathogens	Power (kW)	A_w	Weibull model		Log linear + Shoulder model	
			t_d	t_{5d}	t_d	t_{5d}
Escherichia coli O157:H7	4	0.3	4.59 ± 0.18[a] A	9.21 ± 1.09 A	4.57 ± 0.17 A	12.07 ± 1.30 A
		0.4	4.32 ± 0.31 A	8.65 ± 1.00 A	4.27 ± 0.32 A	11.20 ± 1.30 A
		0.5	4.32 ± 0.73 A	8.10 ± 1.08 A	4.26 ± 0.73 A	10.41 ± 1.08 A
	6	0.3	2.17 ± 0.54 A	7.97 ± 1.35 A	2.22 ± 0.52 A	8.39 ± 1.30 A
		0.4	2.62 ± 0.46 A	6.52 ± 0.47 AB	2.70 ± 0.43 A	7.20 ± 0.78 AB
		0.5	2.04 ± 0.72 A	5.65 ± 0.29 B	2.18 ± 0.69 A	5.89 ± 0.28 B
Salmonella Typhmiurium	4	0.3	4.75 ± 0.21 A	8.64 ± 1.56 A	4.72 ± 0.22 A	11.46 ± 1.35 A
		0.4	4.09 ± 0.25 AB	7.98 ± 1.02 A	4.05 ± 0.25 B	10.15 ± 1.29 AB
		0.5	3.70 ± 0.57 B	6.91 ± 0.56 A	3.75 ± 0.47 B	8.66 ± 0.57 B
	6	0.3	2.56 ± 0.43 A	8.79 ± 1.83 A	2.54 ± 0.41 A	9.64 ± 2.17 A
		0.4	2.39 ± 0.46 A	6.24 ± 1.21 B	2.56 ± 0.44 A	6.63 ± 1.24 B
		0.5	2.02 ± 0.39 A	4.92 ± 0.09 B	2.26 ± 0.53 A	5.07 ± 0.11 B
Listeria monocytogenes	4	0.3	17.28 ± 15.04 A	31.17 ± 1.79 A	6.37 ± 0.21 A	22.33 ± 9.36 A
		0.4	5.03 ± 0.22 A	11.43 ± 0.92 B	5.05 ± 0.26 B	13.80 ± 6.21 A
		0.5	4.55 ± 0.21 A	8.49 ± 1.14 C	4.52 ± 0.22 C	11.15 ± 1.46 A
	6	0.3	3.34 ± 0.83 A	9.44 ± 1.89 A	3.75 ± 0.05 A	9.44 ± 0.55 A
		0.4	2.90 ± 0.53 A	6.69 ± 0.43 B	2.96 ± 0.51 B	7.52 ± 0.71 B
		0.5	2.97 ± 0.25 A	5.68 ± 0.22 B	3.03 ± 0.18 B	6.23 ± 0.34 C

[a] Mean values ± standard deviations. Means with the same upper case letter in the same column per pathogen and microwave heating level are not significantly different ($P > 0.05$).

II-2.4. Discussion

Peanut butter has recently become an important issue in the area of food safety due to several large outbreaks (CDC, 2007a; 2010; 2012) and difficulties of pasteurization of this product. The main cause of peanut butter illness outbreaks is *Salmonella* contamination. Many researchers attempted to pasteurize peanut butter by conventional heating. Shachar and Yaron (2006) reported that conventional heating at 80 and 90°C for 50 min reduced *Salmonella* Agona, *Salmonella* Enteritidis and *S.* Typhimurium by 3.0 log CFU/g. Ma et al. (2009) and He et al. (2011) also used conventional heating to reduce *Salmonella* Tennessee and *Salmonella enterica* but it was not effective as I mentioned above. The long treatment time required by conventional heat pasteurization is one of the main causes of food quality deterioration. Many food ingredients are heat sensitive, so conventional heating induces chemical and physical changes in foods (Chemat et al., 2011). For these reasons new intervention strategies for pasteurization of peanut butter are needed. Recently, Song and Kang (2016a) reported that

microwave heating can be used as a pasteurization method for peanut butter. In this study, I evaluated the effect of a_w of peanut butter on inactivation of pathogens in peanut butter. *E. coli* O157:H7 has not been frequently linked to peanut butter outbreaks but *E. coli* O157:H7 has been implicated in several outbreaks involving low a_w foods (CDC, 2011a; Williams et al., 2000). Kenney and Beuchat (2004) reported that *L. monocytogenes* can survive in 0.33 a_w peanut butter for 24 weeks. Based on these studies, I also confirmed the effect of a_w of peanut butter on inactivation on *E. coli* O157:H7 and *L. monocytogenes* by microwave heating.

Both temperature and microbial inactivation of microwave heat-treated peanut butter of different a_w increased as power, treatment time and a_w increased. A_w and water content of foods are two key factors of thermal treatment which greatly affect temperature rise and microbial inactivation. Jeong and Kang (2014) reported that the rate of temperature increased by radio-frequency heating of 12.6% moisture content red pepper was lower than that of 19.1% moisture content red pepper. He et al. (2013) reported that increased a_w of peanut butter resulted in reduced thermal resistance of

Salmonella enterica. The results from this study show a similar trend. Increasing a_w of peanut butter resulted in higher temperature increase and greater reduction of pathogens.

Microwave heating experiences different starting and ending treatment temperatures and thus usually can-not maintain an isothermal condition. In the case of non-isothermal conditions, inactivation of pathogens in foods can-not be characterized by the Log-Linear Regression model. From this reason, several non-log-linear models have been proposed to explain the survival curves of pathogens, such as the Weibull model (Mafart et al., 2002), Log-Linear + Shoulder or Tail model (Geeraerd et al., 2000), Log-Logistic model (Chen and Hoover, 2003), and Biphasic model (Cerf, 1977). In this study, the Weibull and Log-Linear + Shoulder models were selected to explain the survival curves of the three pathogens because their survival curves did not describe a straight line. These two models achieved better statistical results than did other non-log-linear models in my preliminary studies. The Log-Linear + Shoulder model showed a higher mean R^2 value (0.951) than that of the Weibull model (0.945) and MSE values of these two

models were similar (0.045 and 0.044); therefore the Log-Linear + Shoulder model provided the best fit for microbial inactivation by microwave heating and was especially applicable because inactivation curves of the pathogens in peanut butter by microwave heating showed a shouldering effect. Several studies have confirmed that a shouldering effect occurs during microwave heating (Goldblith and Wang, 1967; Papadopoulou et al., 1995). Valero et al. (2014) also used the Log-Linear + Shoulder model to explain microbial inactivation by microwave heating.

Usually, under non-isothermal conditions, t_d value is used to the resistance of a pathogen to thermal treatment. In this study, I calculated t_d values of the three pathogens in peanut butter of different a_w based on the Weibull and Log-Linear + Shoulder models. Several studies have measured the D-values of pathogens in peanut butter subjected to conventional heating. Ma et al. (2009) reported that the D-value of a cocktail of 5 *Salmonella* strains of non-Tennessee serotypes in 0.45 a_w peanut butter was 9.4 min at 90°C. He et al. (2011) reported that D-values of 5 strains of *Salmonella enterica* in different peanut butters (0.4 a_w) ranged from 2.33 to 3.55 min at

90°C. Also, D-values of *S.* Typhimurium in low-fat and regular formulation 0.4 a_w peanut butter ranged from 2.43 and 1.35 min at 90°C (He et al., 2013). In this study, t_d values of the three foodborne pathogens treated with 6 kW microwave heating were 2.02 to 3.34 min based on the Weibull model. In the case of the Log-Linear + Shoulder model, t_d values of pathogens treated with 6 kW microwave heating ranged from 2.18 to 3.75 min. Decimal reduction times (D-value and t_d value) of 6 kW microwave heat-treated pathogens were similar to those of 90°C conventional heating. But t_{5d} values were different from those of conventional heating. He et al. (2013) reported that calculated minimum times to achieve 5 log reduction of *S.* Typhimurium in 0.4 a_w regular formulation peanut butter based on the Weibull model were 23.83 min by conventional heating at 90°C. Six kW microwave heating of 0.4 a_w peanut butter resulted in t_{5d} values of the three pathogens ranging from 4.92 to 9.44 min. Especially 6 kW microwave heating can reduce *S.* Typhimurium by 5 log after about 5 min. He et al. (2013) also reported that increased a_w reduced the heat resistance of *Salmonella*. But, a_w of peanut butter treated with microwave heating

generally did not affect t_d values of pathogens. Four out of a total of 12 cases showed a significant difference in t_d values. However, increased a_w generally affected t_{5d} values of pathogens (in 8 of 12 cases). In the case of 6 kW microwave heating, increased a_w resulted in less heat resistance of pathogens when I calculated t_{5d} values.

These results indicate that microwave heating can be used as a pasteurization intervention for peanut butter. Inactivation curves of the three pathogens fit well to the Weibull and Log-Linear + Shoulder models and I found that the Log-Linear + Shoulder model gave the best fit for peanut butter pasteurization by microwave heating. t_d values of the three pathogens were similar to D-values for conventional heating but t_{5d} values were dramatically lower than those of conventional heating. Generally, a_w of peanut butter affected t_{5d} values of microwave heating but did not influence t_d and *L. monocytogenes* showed the most resistance to microwave heating, whereas *S.* Typhimurium showed less resistance than the other two pathogens. Results of the present study can be used to optimize microwave heating as a pasteurization intervention for peanut butter.

II-3. A comparison of the effect of 2,450 and 915 MHz microwave heating on the inactivation of foodborne pathogens in large scale peanut butter processing

II-3.1. Introduction

Peanuts are one of the most widely consumed foods worldwide and contains compounds beneficial to health, such as monounsaturated fats, polyphenols, resveratrol, flavonoids, antioxidants, tocopherol, phytosterols and phospholipids (Isanga and Zhang, 2007). Peanut butter is one of the most easily consumed forms of this healthful food. Peanut butter production increased from 1999 to 2008 while candy and snack production has not (USDA, 2010). Peanut butter is usually pasteurized by conventional heating at 70 to 75°C (Ha et al., 2013b). But, there have been several studies which confirmed that conventional heating is not suitable for pasteurization of peanut butter. He et al. (2011) used conventional heating at 72°C for 60 min to reduce *Salmonella enterica* in peanut butter, however, less than 2 log reductions occurred. Ma et al. (2009) reported that conventional thermal treatment at 71°C for 50 min reduced *Salmonella* Tennessee by just 2 log CFU/g. Inadequacy of conventional peanut butter pasteurization of has been manifested in several outbreaks due to *Salmonella* contamination as I

mentioned in Chapter I.

To date, some studies have described the effect of conventional heating on inactivation of *Salmonella* in peanut butter (He et al., 2011, 2013; Ma et al., 2009; Li et al., 2014; Shachar and Yaron, 2006). But most of these studies used methods which are not suitable for industry. They tested only a small amount (Ma et al., 2009; Li et al., 2014) or thin layer of peanut butter to increase the efficacy of conventional heating (He et al., 2011, 2013; Li et al., 2014, Ma et al., 2009). Generally, peanut butter is packaged in cylinder jars containing several hundred grams of product. Jiao et al. (2014) reported that 6 kW 27.12 MHz radio frequency increased the temperature of 460 g of peanut butter to 70°C in 6 min 30 s. A pasteurization intervention for peanut butter of this scale is needed.

Microwaves are part of electromagnetic spectrum which offer many advantages for food processing. Microwaves do not need an intermediate heat transfer medium, can penetrate the material directly and produce efficient volumetric heating (Zhu et al., 2007). Microwave frequencies allocated for use by the food industry include 915 and 2,450 MHz and 2,450

MHz microwave heating is usually used in domestic microwave ovens (Datta and Davidson, 2000). The main difference between these two frequencies is wave length. Nine hundred fifteen MHz microwaves produce 0.33 m whereas 2,450 MHz microwaves produce 0.12 m wavelengths in free space (Tanaka et al., 1999). But there have been few studies investigating the different effects of these two frequencies on food processing. Armbruster and Haefele (1975) utilized 2,450 and 915 MHz microwave heating to process foods. They found no significance differences in cooked food quality between 2,450 and 915 MHz, but their study focused on the effect of plastic film covers on microwave heating, not on the difference between the two frequencies.

The object of this study was to compare the effect of 2,450 and 915 MHz microwaves on temperature increase in different size packages of peanut butter and the inactivation of foodborne pathogens in large containers (400 g) of peanut butter and quality changes during treatment were also investigated.

II-3.2. Materials and Methods

Bacterial strains and cell suspension Strains of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890) and *S.* Typhimurium (ATCC 19585, ATCC 19115, ATCC 700408) were obtained from the bacteria culture collection of Seoul National University (Seoul, Republic of Korea) for this study. Stock cultures were kept frozen at –80°C in 0.7 ml of Tryptic Soy Broth (TSB; Difco, BD, Sparks, MD) and 0.3 ml of sterile 50% (V/V) glycerol. Working cultures were streaked onto Tryptic Soy Agar (TSA; Difco, BD), incubated at 37°C for 24 h and stored at 4°C. Each strain of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was cultured in 5 ml TSB at 37°C for 24 h, harvested by centrifugation at 4000 × *g* for 20 min at 4°C and washed three times with sterile distilled water. The final pellets were resuspended in sterile distilled water, corresponding to approximately 10^8-10^9 CFU/ml. Mixed culture cocktails were prepared by blending together equal volumes of each test strain.

Sample preparation and inoculation Experiments were performed using commercially processed creamy peanut butter was purchased at a local grocery store (Seoul, Republic of Korea) and stored at room temperature (22 ± 1˚C). The composition of the peanut butter (in the order listed on the product label) consisted of roasted peanuts, sugar, hydrogenated vegetable oil (cottonseed, soybean and rapeseed oil) and salt. The nutrition facts label indicated 16 g of fat, 7 g of protein, 6 g of total carbohydrate and 150 mg of sodium per each 32 g serving. For inactivation study, 400 g peanut butter samples were placed in sterile 600 ml Pyrex glass beakers. For inoculation, 4 ml of culture was added to the sample and thoroughly mixed for 10 (400 g peanut butter) min with a sterile spoon to ensure even distribution of pathogens. Uniform distribution of inoculum was confirmed by similar log CFU counts (log 5 – 6 CFU/g) on Sorbitol MacConkey agar (SMAC; Difco) (*E. coli* O157:H7), and Xylose Lysine Desoxycholate agar (XLD; Difco) (*S.* Typhimurium) obtained from 1 g sub-samples of inoculated peanut butter taken from randomly selected locations. For temperature measurement, 50, 100, 200 and 400 g peanut butter samples

were placed in sterile 50, 100, 250 and 600 ml Pyrex glass beaker, respectively. And to adjust a_w the same level of inoculate samples, 0.5, 1, 2 and 4 ml of sterile distilled water was added to the sample and thoroughly mixed for 1 (50 and 100 g peanut butter), 5 (200 g peanut butter) or 10 (400 g peanut butter) min with a sterile spoon to ensure uniform a_w throughout the sample. A_w of peanut butter after adjustment was 0.30. Water activity of inoculated peanut butter was measured by an Aqualab model 4TE a_w meter (METER Group, Inc., Pullman, WA).

Microwave heating treatment Microwave treatment (915 MHz, 6 kW) was performed in a previously described apparatus (Sung and Kang, 2014) and a domestic microwave oven (2,450 MHz, 700 W) (RCD-B41; Rinnai Korea corp., Incheon, Republic of Korea). The sample-filled beaker was placed at the center of the turntable. For temperature measurements, the geometric center temperature of non-inoculated samples of varying quantity in different size beakers was measured by a fiber optic sensor (FOT-L; FISO Technologies Inc., Quebec, Canada) connected to a signal conditioner (TMI-

4; FISO Technologies Inc., Quebec, Canada). For the inactivation study, 400 g of inoculated peanut butter was placed in a 600 ml Pyrex beaker and subjected to microwave heating at 6 kW (915 MHz) for up to 90 s and 700 W (2,450 MHz) for up to 160 s.

Bacterial enumeration After microwave heating treatment, 25 g samples were collected at 4 locations; 1: top side, 2: top center, 3: bottom side and 4: bottom center. Collected samples were mixed with 25 ml of 0.2% PW. Then, the sample and 0.2% PW mixture were diluted in 200 ml of sterile 0.2% PW and homogenized for 2 min in a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1 ml sample aliquots were 10-fold serially diluted in 9 ml of sterile 0.2% PW, and 0.1 ml of sample or diluent was spread-plated onto SMAC and XLD for the enumeration of *E. coli* O157:H7 and *S.* Typhimurium, respectively. Where low populations of surviving cells were anticipated, 1 ml aliquots of the original homogenate were equally distributed between four plates and spread-plated. All plates were incubated at 37°C for 24 h and colonies were

counted.

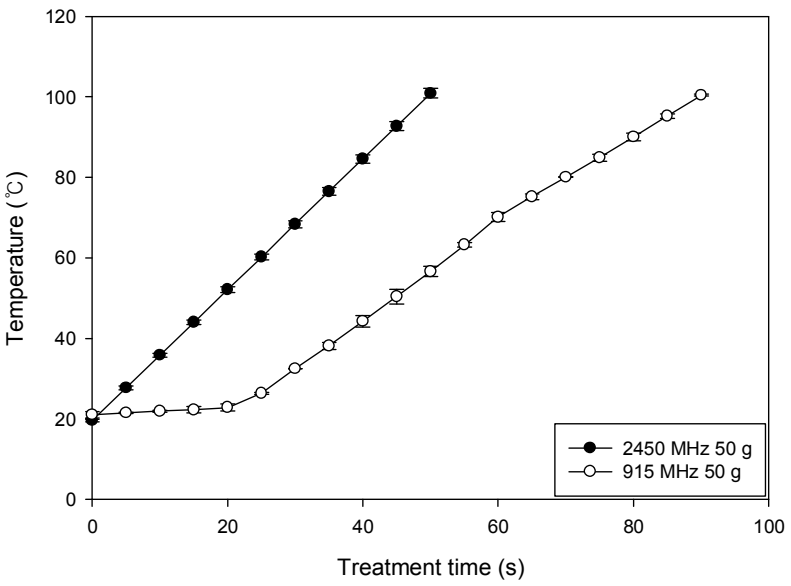
Acid value, peroxide value and color measurement After 90 s (915 MHz) or 160 s (2,450 MHz) of microwave heating treatment, acid and peroxide values of peanut butter were measured as indicators of lipid oxidation. Acid value titrations were determined according to the American Oil Chemists' Society (AOCS) Cd 3d-63 procedure (1998). Peroxide values were determined by iodometric titration according to AOCS Ja 8-87 (2009). Acid value is milligrams of potassium hydroxide necessary to neutralize the free acid in 1 g of sample and peroxide value is the amount of peroxide oxygen per 1 kg of sample. Also, Hunter's color values (*L*, *a* and *b*) were measured using a Minolta colorimeter (model CR300, Minolta Co., Osaka, Japan) after 90 s (915 MHz) or 160 s (2,450 MHz) of microwave heating. Untreated samples were used as controls. *L*, *a,* and *b* values indicate color lightness, redness, and yellowness of the sample, respectively. Experiments were conducted three times.

Statistical analysis All data were analyzed by one-way ANOVA using the Statistical Analysis System (SAS Institute, Cary, NC, USA) and Duncan`s multiple range test to determine if there were significant differences ($P < 0.05$) in mean values. Microbial counts were transformed to $\log_{10}$ values prior to analysis.

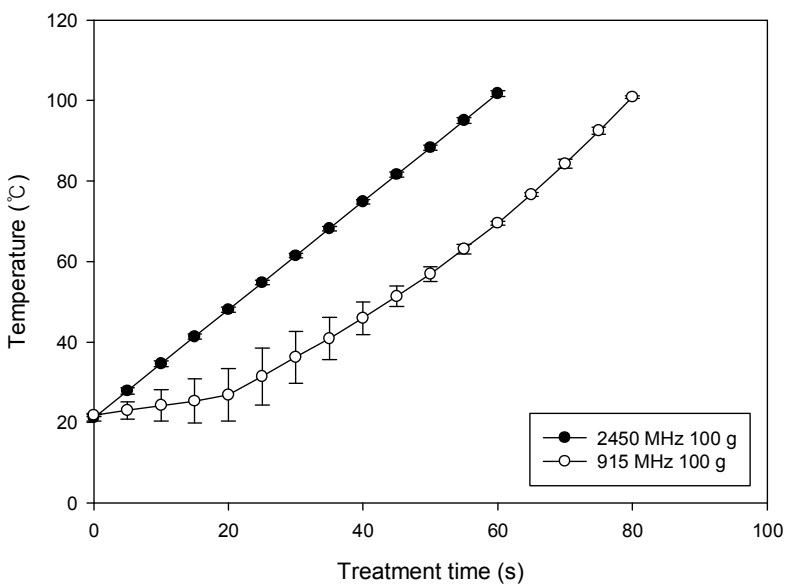
II-3.3. Results

Average center temperatures of peanut butter of varying mass are shown in Fig. II-6. The time required to increase the center temperature of different size peanut butter samples to 100°C was different when treated with 2,450 MHz microwaves. In the case of 50 g peanut butter samples, the center temperature of peanut butter reached 100°C at 50 s. But this temperature was attained only after 60 s (100 g peanut butter), 90 s (200 g peanut butter) and 160 s (400 g peanut butter), respectively, when treated with 2,450 MHz microwaves. But in the case of 915 MHz microwave-treated peanut butter samples, there was little difference in the time needed for the center temperature to reach 100°C. This occurred after 90 s (50 and 400 g peanut butter) and 80 s (100 and 200 g peanut butter), respectively.

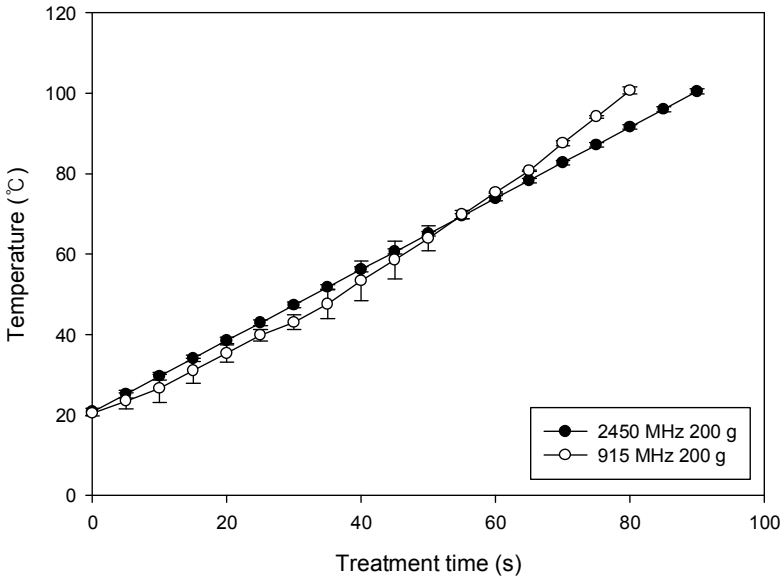
(a)


Temperature (℃)
Treatment time (s)
2450 MHz 50 g
915 MHz 50 g
0
20
40
60
80
100
120

(b)


Temperature (℃)
Treatment time (s)
2450 MHz 100 g
915 MHz 100 g
0
20
40
60
80
100
120

(c)



(d)

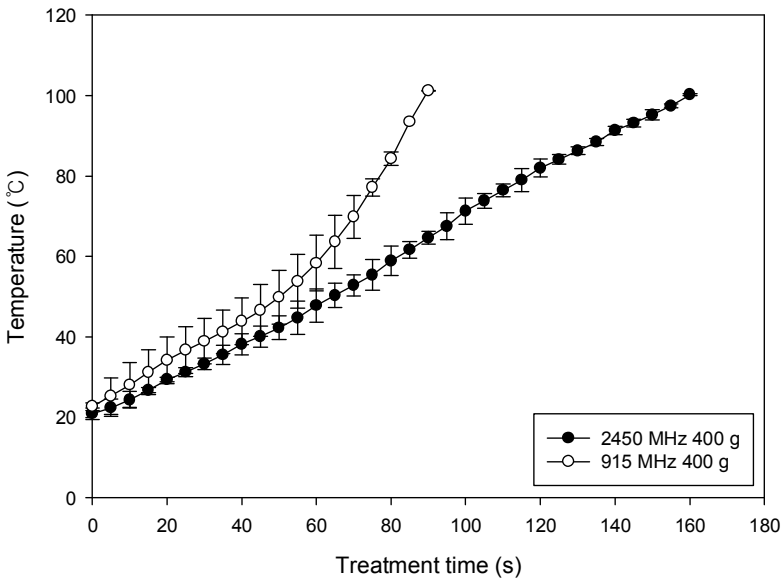


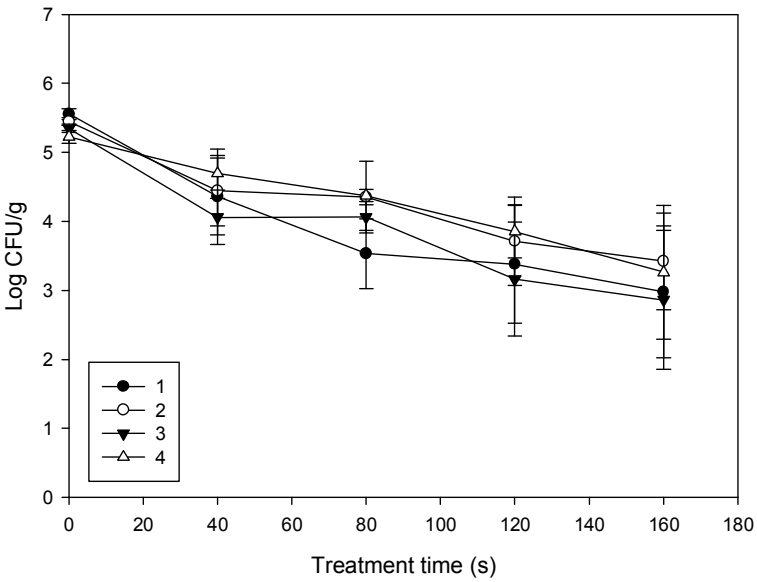
Figure II-6. Center temperature history of different size peanut butter samples treated with 2,450 MHz (●) and 915 MHz (○) microwave heating. (a) 50 g peanut butter, (b) 100 g peanut butter, (c) 200 g peanut butter, (d) 400 g peanut butter.

Reductions in the viable counts of *E. coli* O157:H7 and *S.* Typhimurium cells in peanut butter of different sample size during microwave heating at different frequencies are shown in Fig. II-7 and II-8. The survival curves of *E. coli* O157:H7 and *S.* Typhimurium subjected to 2,450 MHz microwave heating of peanut butter in large scale (400 g) containers is shown in Fig. II-7. Two thousand four hundred fifty MHz microwave heating inactivated populations of *E. coli* O157:H7 in peanut butter by 0.53 to 1.29 (40 s), 0.85 to 2.02 (80 s), 1.37 to 2.18 (120 s) and 1.96 to 2.57 (160 s) log CFU/g, respectively (Fig. II-7a). In the case of *S.* Typhimurium, 2,450 MHz microwave heating reduced populations by 0.18 to 0.55 (40 s), 0.58 to 1.44 (80 s), 1.17 to 2.64 (120 s) and 1.59 to 3.24 (160 s) log CFU/g, respectively (Fig. II-7b).

Fig. II-8 shows survival curves of both pathogens in peanut butter treated with 915 MHz microwave heating. Heating with this wavelength inactivated populations of *E. coli* O157:H7 in peanut butter by 0.93 to 1.58 (25 s), 1.68 to 2.46 (50 s), 2.81 to 3.61 (75 s) and 4.52 to > 4.95 (90 s) log CFU/g, respectively (Fig. II-8a). In the case of *S.* Typhimurium, 915 MHz

microwave heating reduced cell counts by 0.38 to 0.87 (25 s), 1.88 to 2.21 (50 s), 2.86 to 3.33 (75 s) and 3.73 to > 4.10 (90 s) log CFU/g, respectively (Fig. II-8b). Generally, 915 MHz microwave treatment more effectively inactivated pathogens in peanut butter than did 2,450 MHz. Especially, the two pathogens in peanut butter at location 1 were reduced by > 4.95 (*E. coli* O157:H7) or > 4.10 (*S.* Typhimurium) log to under the detection limit (1.0 log CFU/g) when treated with 915 MHz microwave heating for 90 s, but these two pathogens in peanut butter at location 1 were reduced by 2.57 (*E. coli* O157:H7) and 3.24 (*S.* Typhimurium) log CFU/g, respectively, when 2,450 MHz microwave heating was employed.

(a)



(b)

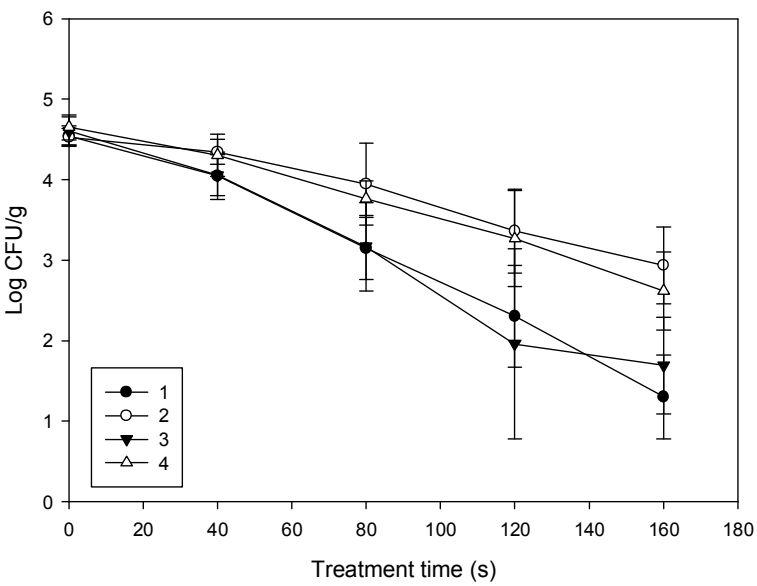
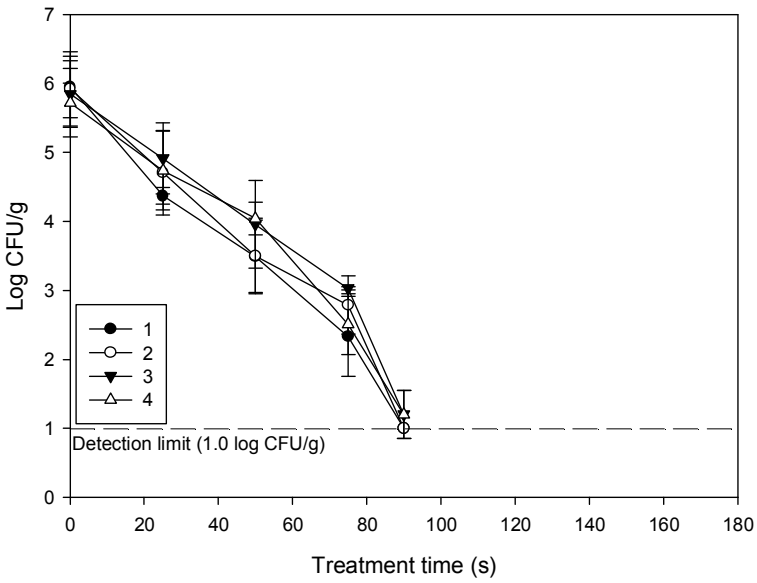


Figure II-7. Inactivation of *E. coli* O157:H7 and *S.* Typhimurium in large scale (400 g) containers of peanut butter at 4 different locations treated with 2,450 MHz microwave. (a) *E. coli* O157:H7; (b) *S.* Typhimurium.

(a)



(b)

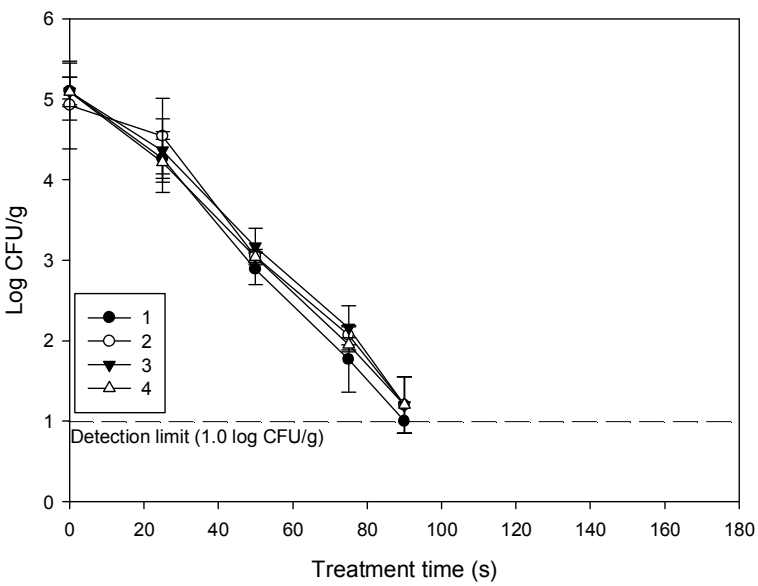


Figure II-8. Inactivation of *E. coli* O157:H7 and *S.* Typhimurium in large scale (400 g) containers of peanut butter at 4 different locations treated with 915 MHz microwave. (a) *E. coli* O157:H7; (b) *S.* Typhimurium.

In this study, acid and peroxide values were not affected by 915 MHz microwave heating (Table II-9). Acid and peroxide values of microwave heat-treated samples were not significantly different from those of non-treated samples. Peroxide values slightly increased after microwave heating but these differences were not significant ($P > 0.05$). However, 2,450 MHz microwave heating changed acid and peroxide values of peanut butter. After 2,450 MHz microwave heating, acid and peroxide values increased significantly ($P < 0.05$).

L, a, and *b* values of 915 MHz microwave-treated samples were not significantly different from those of non-treated samples (Table II-10). *L, a* and *b* values were decreased after microwave heating, but these values were not significantly different from those the of control ($P > 0.05$). Conversely, 2,450 MHz microwave heating changed color values of peanut butter. After 2,450 MHz microwave heating, color values were significantly decreased ($P < 0.05$).

Table II-9. Acid and peroxide values of 2,450 and 915 MHz microwave heat treated peanut butter.

Treatment	Acid value (mg KOH/g)	Peroxide value (mEq/kg)
Control	2.09 ± 0.04[a] A	6.21 ± 1.20 A
2,450 MHz	2.31 ± 0.10 B	9.06 ± 0.26 B
915 MHz	2.04 ± 0.11 A	6.47 ± 0.41 A

[a] Mean of three replications ± standard deviation. Values followed by the same letters within the column are not significantly different (P > 0.05).

Table II-10. Hunter's color values of 2,450 and 915 MHz microwave heat treated peanut butter.

Treatment	Parameter		
	L	*a*	*b*
Control	46.41 ± 1.30[a] A	8.13 ± 0.29 A	25.34 ± 0.92 A
2,450 MHz	43.05 ± 1.43 B	7.65 ± 0.14 B	22.82 ± 0.39 B
915 MHz	44.49 ± 0.46 AB	7.80 ± 0.02 AB	24.38 ± 0.22 A

[a] Mean of three replications ± standard deviation. Values followed by the same letters within the column are not significantly different (P > 0.05).

II-3.4. Discussion

Many researchers have attempted to pasteurize peanut butter with conventional heating (He et al., 2011, 2013; Ma et al., 2009; Li et al., 2014; Shachar and Yaron, 2006) but, pasteurization of large size containers of peanut butter by conventional heating requires excessive time because of the slow rate of conductive heat transfer from the outside to inside of this food product. Long exposure to heat is one of the main reasons for food quality deterioration. Conventional heating causes adverse chemical and physical changes in foods because many food ingredients are heat sensitive (Chemat et al., 2011). Also, as I mentioned above, studies on pasteurization of peanut butter have generally used only small amounts (several g) and/or thin layers of peanut butter. But in industry, manufacturers package peanut butter into cylindrical jars in quantities usually exceeding 400 g. Thus, a control intervention for large scale production of peanut butter is needed. Recently, Song and Kang (2016a, 2016b) reported that microwave heating can be used as a control intervention for peanut butter. In this study, I evaluated the

effect of microwave frequency on temperature increase of peanut butter and inactivation of pathogens in large size containers of peanut butter (400 g). In last decade, there have been several large outbreaks due to *Salmonella* contaminated peanut butter (Cavallaro et al., 2011; CDC, 2007a, 2012). Additionally, survival of *E. coli* O157:H7 in low water activity foods has been reported (Blessington et al., 2012; Kimber et al., 2012). Because of these reasons, I used *S.* Typhimurium and *E. coli* O157:H7 in this study.

Product size is a one of the factors which affect temperature increase during microwave heating. In this study, I used 50, 100, 200 and 400 g of peanut butter for comparison of the effect of microwave frequency. When different quantities of peanut butter were treated with 2,450 MHz microwaves, the time for the center temperature to come up to 100ºC increased along with sample size. In the case of 50 g samples, the center temperature reached 100°C after 50 s. But, the time needed for center temperature of 100 g and 200 g peanut butter samples to reach this temperature was 60 s and 90 s, respectively. One of the main reasons for this phenomenon is penetration depth. The penetration depth of a wave is

proportional to its wavelength, thus, a 915 MHz microwave produces deeper penetration depth than a 2,450 MHz microwave. Accordingly, in 2,450 MHz microwave treated peanut butter samples, the outside of the peanut butter mass was more easily heated by microwaves than the inside and the heat generated by microwaves was transferred to the inside of the peanut butter by conduction. Microwaves generate volumetric heating which means that they generate heat inside of foods. Heat transfer by conduction is much slower in peanut butter than by volumetric heating. When heating small containers of peanut butter, a 2,450 MHz microwave system is usable because of the small depth of these packages. But, when scaling up into larger containers, 2,450 MHz microwave can not penetrate to the inside of the peanut butter and heat generated at the outside of the peanut butter mass transfers slowly to the inside of peanut butter. This becomes especially problematic considering the increased diameter of jars used to package larger quantities of peanut butter.

When using thermal treatment for the inactivation of pathogens in food, heat distribution is important. With conventional heating, heat transfers to

the inside of food by conduction or convection. Heat transfer by conduction and convection, resulting in non-uniform heat distribution. This non-uniform heat distribution can result in a partial pasteurization effect necessitating longer treatment time (the temperature of the cold spot increasing slowly) and longer treatment time can result in quality deterioration of foods such as surface burden. Because of these disadvantages of conventional heating, researchers have focused on non-thermal inactivation methods to control *Salmonella* in peanut butter. Ban and Kang (2014) used gamma irradiation to inactivate *S.* Typhimurium in peanut butter. Three kGy of gamma irradiation reduced this pathogen by 3.5 to 4.0 log CFU/g. D'Souza et al. (2012) used high hydrostatic pressure under various conditions (400 to 600 MPa for 4 to 18 min) to reduce cell numbers of a *Salmonella enterica* serovar cocktail in creamy peanut butter. This treatment resulted in 1.6 to 1.9 log CFU/g reduction of *Salmonella*. Microwave processing is a volumetric heating methodology which has the advantage on uniform heat distribution and inactivation effect. In this study 915 MHz microwaves showed a uniform antimicrobial effect and pathogen

inactivation did not show position-dependence. At all 4 locations, pathogen reductions were similar and showed no significant differences ($P > 0.05$). Also, reductions of pathogens at 4 different locations by 2,450 MHz treatment displayed no significant difference, but this is due to the large standard deviations. Reductions of pathogens at locations 1 and 3 were larger than those of position 2 and 4. Positions 1 and 3 are on the outside of the peanut butter samples and position 2 and 4 are in the sample center. This result also shows that 2,450 MHz microwave can not penetrate deeply into peanut butter, as I mentioned previously. Two thousand four hundred fifty MHz microwave did not penetrate into the peanut butter interior and only applied heat to the outer portion. This position-dependent inactivation effect is not the only problem of 2,450 MHz microwave heating. As shown in Fig. II-7, the pathogen reductions have large standards deviation. This result is based on three experimental replicates; however, I actually performed five replicates, but the deviations were still not reduced even after replicating five times (data not shown). Therefore, I used results from three replicates in this study. Large deviations mean that inactivation of pathogens in peanut

butter by 2,450 MHz microwaves have very poor reproducibility and can not be used by industry. Nine hundred fifteen MHz microwave heating is much suitable for industrial application.

For high lipid-containing foods like peanut butter, lipid oxidation is a major cause of quality deterioration. Usually, lipid oxidation results from exposure to high temperature but non-thermal treatment also can induce lipid oxidation. Albertos et al. (2017) used dielectric barrier discharge (DBD) plasma on mackerel fillets. After treatment, levels of peroxides and dienes increased. Jayasena et al. (2015) also reported that DBD plasma treatment on pork butt and beef loin resulted in increasing TBARS values. Acid and peroxide values are usually used as indicators of lipid oxidation of foods. In this study, since I used microwave heating as a control intervention, I also investigated acid and peroxide values of peanut butter. Acid and peroxide values did not change during 915 MHz microwave heating but these values increased after 2,450 MHz microwave treatment. Thus, 2,450 MHz microwave heating induced lipid oxidation of peanut butter. Also, 2,450 MHz microwaves induced color changes. After 2,450 MHz microwave

treatment, surface burden occurred. But the color changes were not large, because I measured color values after mixing with a spoon. These color changes were also detected when small amount of peanut butter (50 g) was treated by 2,450 MHz microwave (data not shown).

In conclusion, 915 MHz microwaves are much more suitable as a control intervention for peanut butter than 2,450 MHz microwaves. Nine hundred fifteen MHz microwave heating showed excellent inactivation of pathogens was uniform and did not affect peanut butter quality. But 2,450 microwave heating reduced pathogens less than 915 MHz, showed non-uniform pathogen inactivation and affected food quality. Also, many published peanut butter pasteurization investigations used sample sizes, but in my study, I demonstrated that pasteurization of large peanut butter containers is feasible and suitable for industry. Nine hundred fifteen MHz microwave heating could be an alternative control intervention for the peanut butter processing industry.

II-4. Inactivation of foodborne pathogens on nuts by vacuum-sealed dry heat treatment

II-4.1. Introduction

People have enjoyed consuming nuts since prehistoric times. In recent years, nuts have attracted attention because consumers are increasingly interested in health and nutrition. Nuts contain phytochemicals including carotenoids, phenolic acids, phytosterols and polyphenolic compounds (Bolling et al., 2011). Also, nut consumption reduces type 2 diabetes, cardiovascular disease and numerous other cases of mortality (Luo et al., 2014). Almonds are one of the most recognizable nuts in the world, accounting for 28% of the nut market in 2011 (Jeong et al., 2017).

In March, 2007, the USDA mandated that almonds be processed to reduce *Salmonella* by a minimum of 4 log (ABC, 2007a). The USFDA approved oil roasting, dry roasting, blanching, steam processing and propylene oxide (PPO) as pasteurization methods suitable for almonds (USDA, 2007). Drying roasting of almonds is the most commonly used method in almond processing. But, according to recommendations from the Almond Board of California, in order to reduce *S.* Enteritidis PT 30 by > 4 log, 100 min of

treatment at 121.1°C is needed (ABC, 2007b). Although this step will satisfy people who enjoy roasted almonds. Processing at this time and temperature results in 'roasted' almonds which means that drying roasting is not a suitable control intervention for raw almond processing. Furthermore, in order to kill *S.* Enteritidis PT 30, processors can-not regulate the degree of roasting. Among FDA approved pasteurization methods, PPO is the only non-thermal treatment but PPO is not approved in Europe because of harmful residues remaining on almonds (Cornucopia Institute, 2007). Blanching uses hot water for a short time but drying interval is required. Because of these numerous disadvantages of FDA approved methods, researchers are focusing on non-thermal pasteurization interventions. Niemira (2012) used cold plasma to reduce pathogens on almonds. But 20 s of cold plasma treatment reduced *E. coli* O157:H7 and *Salmonella* only by about one log CFU/g. Lacombe et al. (2016) reported that 405 nm monochromatic blue light for 10 min reduced *S.* Enteritidis PT 30 by 0.5 to 1.26 log CFU/g. These two studies were not able to demonstrate 4 log reduction of *S.* Enteritidis PT 30. A new intervention for almond

pasteurization is needed.

Dry heat is a type of conventional heating which processes foods in a hot oven and is usually used for medical devices (Klein, 2005). In the food science field, dry heat is usually used for inactivation of foodborne pathogens in seed (Bang et al., 2011a, 2011b; Feng et al., 2007; Neetoo and Chen, 2011). But the biggest disadvantage of dry heat application to foods is longer treatment time. Foods are usually heat sensitive and this reason leads people use dry heat treatment at low temperature. Bang et al. (2011a and 2011b) used 55, 60, 70 and 80°C for 48 h, Neetoo and Chen (2011) used 55 to 70°C for 8 days and Feng et al. (2007) used 55°C for 8 days. To reduce treatment time, dry heat is usually combined with other control interventions (Bang et al., 2011a, 2011b; Neetoo and Chen, 2011). An improved method to increase the effect of dry heat and reduce treatment time is needed.

Therefore the objectives of this study were to evaluate the influence of packaging methods on dry heat inactivation of foodborne pathogens on almonds and document the color changes following dry heat treatment.

II-4.2. Materials and Methods

Bacterial strains and cell suspension *S.* Enteritidis PT 30 and *S.* Typhimurium DT 104 were obtained from the bacteria culture collection of Seoul National University (Seoul, Republic of Korea) and *S.* Senftenberg KVCC 0590 was obtained from the Korea Veterinary Culture Collection (KVCC; Anyang, Republic of Korea) for this study. Stock cultures were prepared by growing culture transfers in 5 ml of tryptic soy broth (TSB; Difco, BD, Sparks, MD) at 37°C for 24 h. Seven-tenth ml of broth culture was combined with 0.3 ml of sterile 50% (v/v) glycerol and kept frozen at –80°C. Working cultures were streaked onto tryptic soy agar (TSA; Difco, BD), incubated at 37°C for 24 h and stored at 4°C.

Preparation of inoculum For each experiment, inoculum was prepared individually for each strain using the method described by Danyluk et al. (2005). A loopful from a single isolated colony of each strain of *S.* Enteritidis PT 30, S. Typhimurium, and S. Senftenberg was cultured in 30

ml of TSB at 37°C for 24 h, then a loopful was transferred into 30 ml of TSB, and incubated at 37°C for 18 h. For production of a bacterial lawn, 1 ml of the overnight culture was spread onto each of 5 TSA plates followed by incubation at 37°C for 24 h. The bacterial lawn was dislodged by adding 4.5 ml of 0.2% peptone water (PW; Difco) to each plate and rubbing with a sterile cotton swab. For each *Salmonella* serovars, cell suspensions were collected from the five plates and pooled, corresponding to approximately 10^9 CFU/ml. These final suspensions of the three *Salmonella* strains were used separately in this study.

Sample preparation and inoculation Raw shelled almonds were purchased from a local grocery store (Seoul, Republic of Korea). For inoculation, 15 ml of prepared inoculum (*S.* Enteritidis PT 30, *S.* Typhimurium or *S.* Senftenberg) was added to 500 g samples inside sterile stomacher bags (Labplas, Inc., Sainte-Julie, Quebec, Canada), and then mixed by hand for 1 min. The inoculated samples were dried for 24 h inside a biosafety hood (25°C) with the fan running until the moisture contents of

the samples equaled those of non-inoculated samples (ca. 4.27%, dry basis and a_w 0.30). The final cell concentration was 6 to 7 log CFU/g.

Open, ambient-sealed and vacuum-sealed dry heat treatment In this study, I used 3 different packaging methods. First, open heating, or conventional dry heat treatment, was used. For open heat treatment, 10 g of inoculated almonds were placed in a 90 mm petri dish and the cover was removed during heat treatment. The second was heating of almonds sealed under ambient atmospheric (non-vacuum) pressure (ambient-sealing heat treatment). Ten g of inoculated almonds were placed in aseptic nylon-polyethylene bags (100 x 100 mm) of 0.06 mm film thickness (YH Korea, Seoul, Republic of Korea) and sealed under atmospheric pressure using a sealing machine. Lastly, heating of vacuum-sealed almonds was performed. Ten g of inoculated almonds were similarly placed in aseptic nylon-polyethylene bags and vacuum-packaged with a sealing machine (Airzero, Ansan, Republic of Korea). The three types of packaged samples were moved to an oven (Thermostable^{TM} ON-32, Wisd laboratory instruments)

with a temperature of 90 or 95°C and treated for up to an hour at 15 min increments.

Bacterial enumeration For enumeration of pathogens, each treated sample was immediately transferred to a sterile stomacher bag (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 90 ml of sterile 0.2% PW and homogenized for 2 min in a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1 ml aliquots of homogenized samples were tenfold serially diluted in 9 ml of sterile 0.2% PW, and 0.1 ml of sample or diluent was spread-plated onto Xylose Lysine Desoxycholate agar (XLD; Difco), a selective medium for the enumeration of generic Salmonella, including S. Enteritidis PT 30, S. Typhimurium and S. Senftenberg. Where low populations of surviving cells were anticipated, 1 ml aliquots of the original homogenate were equally distributed between four plates and spread-plated.

Color, moisture content and a_w measurement After treatment,

Hunter's color values (L, a and b) were measured using a Minolta colorimeter (model CR300, Minolta Co., Osaka, Japan). Moisture content of almonds was measured with a Mettler-Toledo HB43-S moisture analyzer (Mettler-Toledo, Columbus, OH) and a_w was measured with an Aqualab model 4TE a_w meter (METER Group, Inc., Pullman, WA) to confirm the evaporation of water.

Statistical analysis All data were analyzed by one-way ANOVA using the Statistical Analysis System (SAS Institute, Cary, NC, USA) and Duncan`s multiple range test to determine if there were significant differences ($P < 0.05$) in mean values of microorganism populations. Microbial counts were transformed to log values prior to analysis. One log was used for calculations in cases where populations were reduced to below the detection limit (1.0 log CFU/g).

II-4.3. Results

Initial populations of *S.* Enteritidis PT 30, *S.* Typhimurium and *S.* Senftenberg on inoculated almonds were 7.54, 7.04 and 6.20 log CFU/g, respectively. Vacuum-sealed dry heat effectively reduced levels of the three *Salmonella* serovars on almonds.

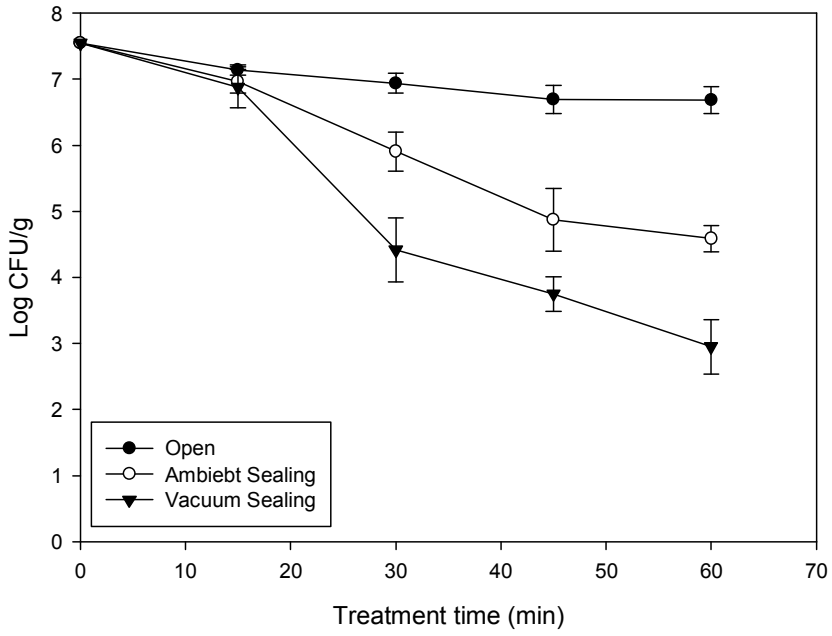
Reductions of *Salmonella* on almonds are shown in Fig. II-9 to 14. Fig. II-9a shows the inactivation effect of 90°C dry heat on *S.* Enteritidis PT 30 in 3 types of packaged almonds. Open heat treatment reduced this pathogen by 0.40 (15 min), 0.60 (30 min), 0.85 (45 min) and 0.86 (60 min) log CFU/g, respectively. Ambient sealing heat treatment inactivated this pathogen by 0.58 (15 min) to 2.96 (60 min) log CFU/g. In the case of heat treated vacuum-sealed almonds, *S.* Enteritidis PT 30 was reduced by 0.68 (15 min) to 4.59 (60 min) log CFU/g.

Fig. II-9b shows the inactivation of *S.* Typhimurium by 90°C heating of three different type of packaged almonds. The reduction trend of this pathogen was similar to that of *S.* Enteritidis PT 30. Open heat treatment

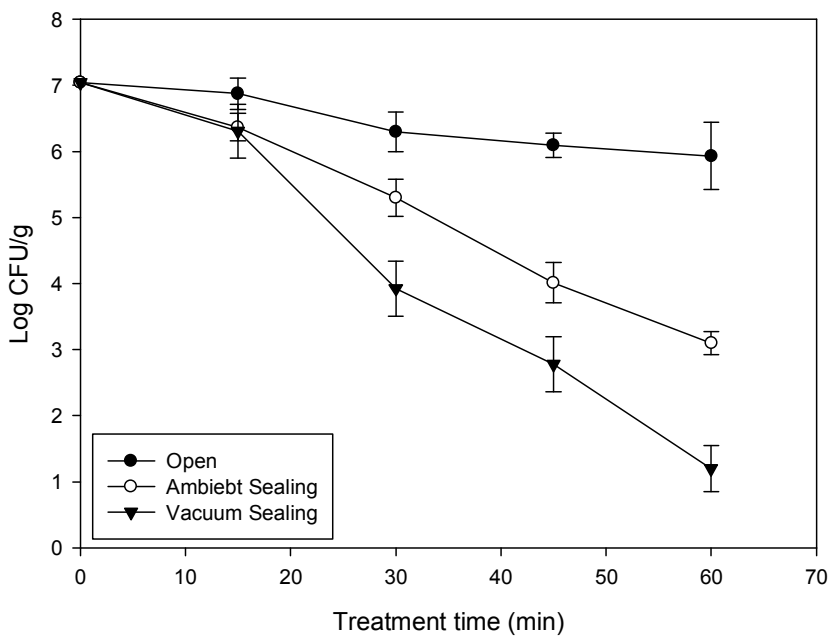
reduced *S.* Typhimurium by 0.17 to 1.11 (60 min) log CFU/g. Ambient sealing heat treatment for 15, 30, 45 and 60 min inactivate this pathogen by 0.67, 1.74, 3.03 and 3.94 log CFU/g, respectively. In the case of heat treated vacuum sealed almonds, this pathogen was reduced by 0.73 (15 min) to 5.84 (60 min) log CFU/g.

Fig. II-9c shows the effect of 3 types of 90°C dry heating on inactivation of *S.* Senftenberg on almonds. The reduction trend of *S.* Senftenberg in peanut butter by dry heat was similar to those of *S.* Enteritidis PT 30 and *S.* Typhimurium. Open heat treatment inactivated this pathogen by 1.33 (15 min) to 2.49 (60 min) log CFU/g. Ambient sealing heat treatment inactivates this pathogen by 1.22 (15 min) to 3.71 (60 min) log CFU/g. In the case of vacuum-sealed almonds heat treated for 15, 30, 45 and 60 min, *S.* Senftenberg was reduced by 1.70, 3.40, > 5.20 and > 5.20 log CFU/g, respectively.

(a)


Log CFU/g
Treatment time (min)
Open
Ambiebt Sealing
Vacuum Sealing

(b)


Log CFU/g
Treatment time (min)
Open
Ambiebt Sealing
Vacuum Sealing

(c)

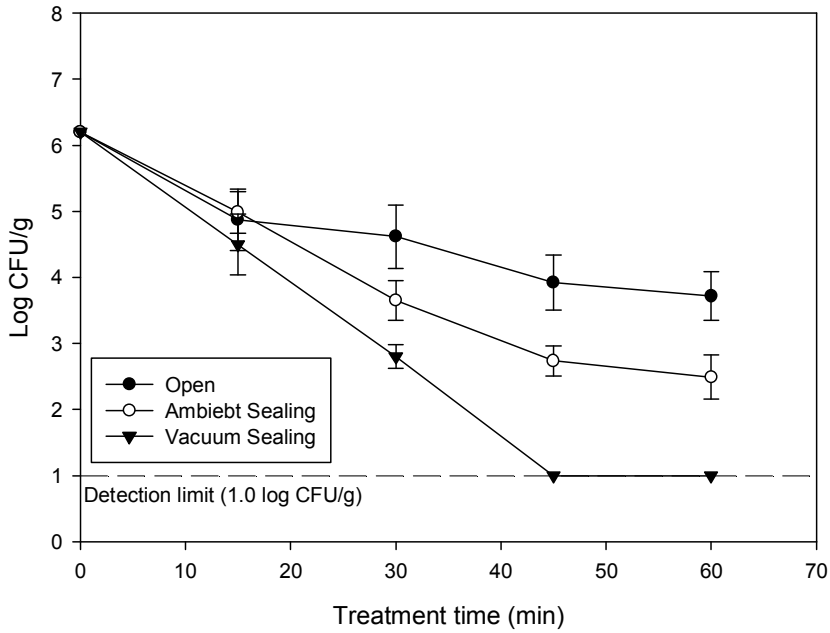


Figure II-9. Survival curves for *Salmonella* on almonds treated with dry heat at 90°C packaged with open (●), ambient sealing (○) and vacuum sealing (▼). (a) *S.* Enteritidis PT 30; (b) *S.* Typhimurium; (c) *S.* Senftenberg.

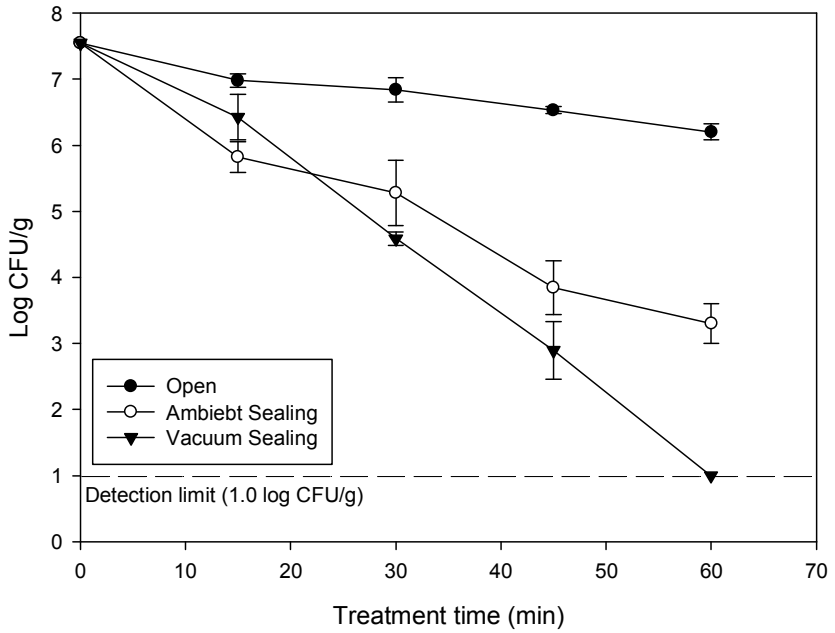
Fig. II-10a shows the inactivation effect of 95°C dry heat and 3 types of packaging on *S.* Enteritidis PT 30 levels on almonds. Open heat treatment reduced this pathogen by 0.56 (15 min), 0.70 (30 min), 1.01 (45 min) and 1.34 (60 min) log CFU/g, respectively. Ambient sealing heat treatment inactivated the pathogen by 1.30 (15 min) to 4.68 (60 min) log CFU/g. In the case of vacuum-sealed heat-treated almonds, *S.* Enteritidis PT 30 was reduced by 1.12 (15 min) to > 6.54 (60 min) log CFU/g.

Fig. II-10b shows 95°C dry heat inactivation of *S.* Typhimurium in three types of packaged almonds. The reduction trend of this pathogen was similar to that of *S.* Enteritidis PT 30. Open heat treatment reduced *S.* Typhimurium by 0.37 (15 min) to 1.25 (60 min) log CFU/g. Ambient sealing heat treatment for 15, 30, 45 and 60 min inactivated this pathogen by 1.15, 2.32, 3.18 and 4.55 log CFU/g, respectively. In the case of vacuum-sealed heat-treated almonds, this pathogen was reduced by 1.34 (15 min) to > 6.04 (60 min) log CFU/g.

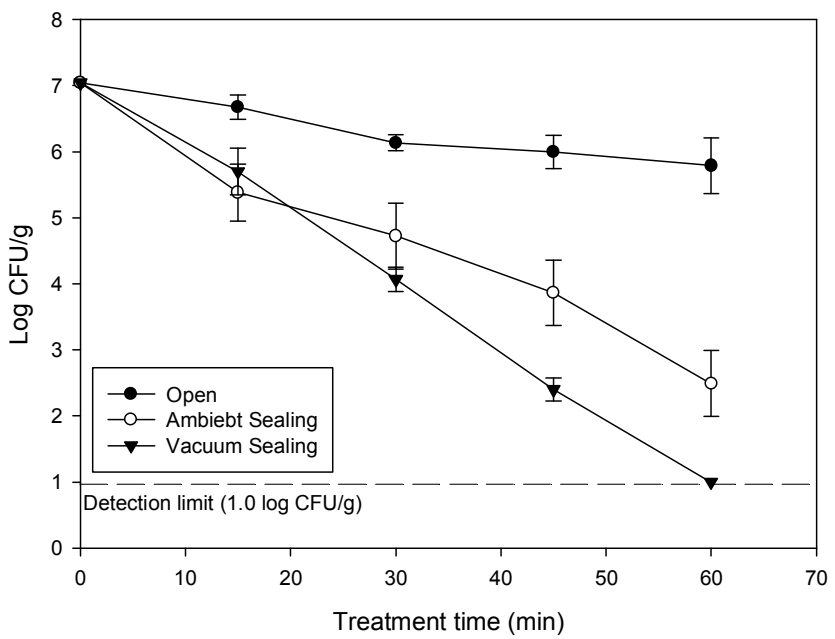
Fig. II-10c shows the effect of 95°C dry heating and the three packaging types on inactivation of *S.* Senftenberg on almonds. The reduction trend of *S.*

Senftenberg on almonds by dry heat was similar to those of *S.* Enteritidis PT 30 and *S.* Typhimurium. Open heat treatment reduced this pathogen by 1.24 (15 min) to 2.20 (60 min) log CFU/g. Sealing heat treatment inactivated this pathogen by 1.84 (15 min) to 4.87 (60 min) log CFU/g. In the case of vacuum-sealed heat-treated almonds for 15, 30, 45 and 60 min, *S.* Senftenberg was reduced by 2.03, 4.32, > 5.20 and > 5.20 log CFU/g, respectively.

(a)


Log CFU/g
Treatment time (min)
Open
Ambiebt Sealing
Vacuum Sealing
Detection limit (1.0 log CFU/g)

(b)


Log CFU/g
Treatment time (min)
Open
Ambiebt Sealing
Vacuum Sealing
Detection limit (1.0 log CFU/g)

(c)

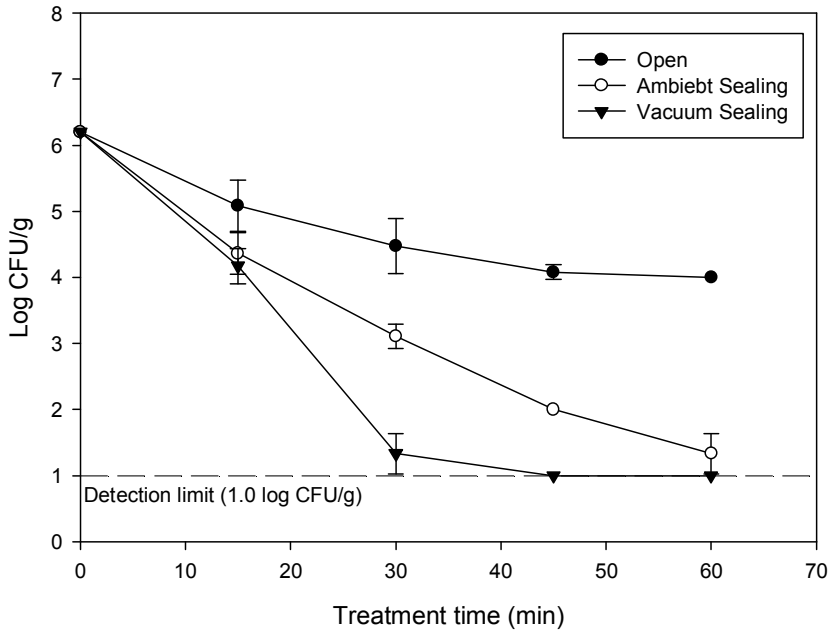


Figure II-10. Survival curves for *Salmonella* on almonds treated with dry heat at 95°C packaged with open (●), ambient sealing (○) and vacuum sealing (▼). (a) *S.* Enteritidis PT 30; (b) *S.* Typhimurium; (c) *S.* Senftenberg.

Table II-11 shows Hunter's color values, moisture content and a_w of non-treated and dry-heated almonds. *L, a,* and *b* values of dry heat-treated samples were not significantly different from those of non-treated samples. Moisture content decreased after open and sealing heat treatment but did not significantly change after vacuum-sealed dry heat treatment. A_w decreased after all kinds of dry heat treatment.

Table II-11. Hunter's color values, moisture content and a_w of almonds subjected to three kinds of packaging and dry heat.

Parameter	Treatment						
	Control	90°C Open	90°C Ambient Sealing	90°C Vacuum Sealing	95°C Open	95°C Ambient Sealing	95°C Vacuum Sealing
L	48.03 ± 2.70[a]	47.60 ± 2.73	48.75 ± 3.43	48.98 ± 3.47	48.38 ± 2.95	47.98 ± 1.45	48.43 ± 3.94
a	16.53 ± 0.87	16.55 ± 0.83	16.80 ± 1.10	17.11 ± 0.31	16.80 ± 0.49	17.59 ± 0.61	17.34 ± 1.08
b	32.17 ± 3.27	29.86 ± 2.95	32.30 ± 1.64	32.27 ± 3.19	32.77 ± 1.99	31.83 ± 1.34	31.08 ± 2.29
Moisture content (%)	4.27 ± 0.04 A	3.13 ± 0.11 C	3.77 ± 0.09 B	4.11 ± 0.08 A	3.10 ± 0.14 C	3.71 ± 0.23 B	4.17 ± 0.01 A
A_w	0.30 ± 0.01 A	0.19 ± 0.00 E	0.24 ± 0.01 C	0.29 ± 0.00 B	0.17 ± 0.01 F	0.23 ± 0.00 D	0.28 ± 0.01 B

[a] Mean of three replications standard deviation. *L, a* and *b* values were not significantly different and moisture contents and a_w followed by the same letters within the row are not significantly different ($P > 0.05$).

II-4.4. Discussion

A number of studies have reported on the inactivation of foodborne pathogens on nuts by dry heat (Bari et al., 2009; Beuchat and Mann, 2011). But, Bari et al. (2009) using dry heat (60°C) for several days and at less than 110°C for 20 min were not able to reduce *Salmonella* in walnuts (Beuchat and Mann, 2011). Conventional heating is the simplest pasteurization method but can not effectively reduce pathogens in low water activity foods. For this reason, many researchers are focusing on other thermal technologies, rather than dry heat, to reduce pathogens in almonds. Yang et al. (2010) used infrared heating to reduce *Enterococcus faecium* strain NRRL B-2354 which is approved by the USDA as a surrogate for *S.* Enteritidis PT 30. Infrared roasting at 130°C effectively reduced this microorganism by 3.56 log but in the process almonds were roasted to a dark color. Harris et al. (2012) reported that hot water treatment at 70°C for 4 min reduced *S.* Enteritidis PT 30 by 3.9 log CFU/g. Sample size was 50 g and BSA (Bismuth sulfite agar) was used as a selective medium for

enumerating *Salmonella*. Chang et al. (2010) used saturated steam as a control method for almonds. They reported that steam treatment effectively reduced *S.* Enteritidis PT 30 but the quality of almonds suffered. Ha and Kang (2015) used a combination of lactic acid spray with near-infrared radiant heating to reduce *S.* Enteritidis PT 30 on almonds. The combination treatment for 5 min reduced this pathogen by 3.9 log CFU/g.

The resistance of pathogens to heat is affected by water content of foods. There are several studies which confirm that heat resistance of *Salmonella* is inversely proportional to water activity. Goepfert et al. (1970) reported that D-values of *Salmonella* Montevideo and *Salmonella* Tennessee in a_w 0.99 sucrose solutions were 1.1 and 0.8 min but those increased to 75 and 35.9 min, respectively, when these pathogens were heated at 57.2°C in a_w 0.87 sucrose solution. This enhanced heat resistance is the main challenge facing low water activity food pasteurization. Moreover, moisture content of food usually decreases during dry heating. Limcharoenchat et al. (2014) reported that during drying heating of almonds, a_w was reduced from 0.54 to 0.22. Dry heating without moisture decrease enhances the inactivation of

foodborne pathogens. Jeong et al. (2009) used moist heat to reduce *S.* Enteritidis PT30 on almonds. Five percent moist heat for 30 min reduced this pathogen by 1.66 log, but 70% moist heat for 80 s reduced *S.* Enteritidis PT30 by 2.50 log CFU/g at 121°C. In this study, I also documented reduced moisture content following dry heating (from 4.27 to 3.13 at 90°C and 3.10 at 95°C). A method to retain water content of foods during dry heating is needed.

Modified Atmosphere Packaging (MAP) is packaging with a modified internal gas composition. MAP is usually used in meat process or quality evaluation studies. Mexis et al. (2009) used MAP for quality evaluation of raw ground almonds; quality factors of ground almonds were found to be affected by atmosphere. Vacuum packaging is one of the MAP methods that remove air from the package prior to sealing. Vacuum packaging has the advantages of maintaining the moisture content of foods and reducing lipid oxidation by removal of oxygen. In this study, *Salmonella* on vacuumed-sealed almonds treated with dry heat was reduced more effectively than on almonds subjected to open or ambient-sealed heating. This is because

vacuum packaging helps almonds preserve their moisture content. The moisture content of almonds treated with vacuum-sealing and dry heat was not significantly different ($P > 0.05$) from the control but those treated with open or ambient-sealing heat were reduced significantly ($P < 0.05$). This evaporation of water affected the heat transfer rate of this food. Thermal conductivity of water (0.58 W/m/°C) is much higher than that of air (0.024 W/m/°C), which leads to faster heat transfer in water (Hong, 2016). Based on the results of this study, the moisture content of almonds was maintained with vacuum packaging, which resulted in a continuous rate of heat transfer to the pathogens.

Thermal resistance of pathogens depends on their strain. In this study, *S.* Enteritidis PT 30 showed the most resistance to thermal treatment, whereas *S.* Senftenberg showed the least resistance. These results correlate with some other studies. Jeong et al. (2017) used radio frequency to reduce *Salmonella* on almonds. *S.* Typhimurium and *S.* Senftenberg on almond surfaces were reduced to under the detection limit at 40 s, but *S.* Enteritidis PT 30 was only reduced by 3.7 log CFU/g at 40 s. Also, *S.* Senftenberg

internalized in almonds was reduced to under the detection limit at 20 s, but *S.* Typhimurium and *S.* Enteritidis PT 30 were only reduced by 2.9 and 2.4 log CFU/g, respectively, at 20 s. Also, Harris et al. (2012) reported the D-value of *S.* Enteritidis PT 30 on almonds as 0.39 whereas that of *S.* Senftenberg 775W on almonds was 0.37 with 88°C hot water treatment. *S.* Senftenberg 775W is one of the most heat resistant *Salmonella* isolates (Goepfert et al., 1970).

Conventional heating usually affects food quality because most foods are heat-sensitive. In this study, I confirmed that dry heat (combined with open-, ambient- or vacuum-sealing) for 1 h did not affect almond color values. Quality changes of nuts during thermal treatment have been reported several times. Chang et al. (2010) used saturated steam treatment to reduce *Salmonella* on almonds. Steam treatment effectively reduced this pathogen but resulted in skin bleaching and detachment of almonds. Wang et al. (2007) used radio frequency for insect control in walnuts. But radio frequency increased the peroxide value of walnuts. As I described previously, vacuum packaging has an advantage of preventing lipid oxidation. I did not confirm

lipid oxidation during open, ambient-sealing and vacuum-sealing heat treatment, but there are several studies which confirm that vacuum packaging prevents lipid oxidation. Nam and Ahn (2003) reported that TBARS values of vacuum-packaged raw turkey breast did not change during 10 days storage at refrigerated temperature. Ahn et al. (2001) reported that TBARS value of vacuum packaged cooked beef was not changed during storage whereas that of aerobic packaged samples increased significantly ($P < 0.05$).

In conclusion, this study evaluated vacuum-sealed dry heat as a novel pasteurization technology for almonds. Vacuum-sealed dry heat treatment effectively reduced *Salmonella* on almonds and did not affect almond color. The pasteurization effect follows this sequence: open < ambient-sealed < vacuum-sealed heating. Vacuum-sealed heating could be utilized as a control intervention by the almond industry. However, further investigations need to be performed to confirm the effect of different packaging methods combined with dry heat on the quality of almonds.

Chapter III.

Inactivation of foodborne pathogens in foods by non-thermal treatment

A part from this chapter were published in

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III-1. Inactivation of foodborne pathogens in apple juice at different pH levels by gaseous ozone treatment

III-1.1. Introduction

Enterohemorrhagic *Escherichia coli* O157:H7 causes hemorrhagic colitis, and can lead to hemolytic uremic syndrome which produces symptoms such as hemolytic anemia, thrombocytopenia and renal injury (Rangel et al., 2005; Reiss et al., 2006). *Salmonella enterica* serovar Typhimurium causes nontyphoidal salmonellosis and typically presents itself as a self-limiting gastroenteritis (Boyle et al., 2007). *Listeria monocytogenes* is generally known as a pathogen which causes bacteremia, meningoencephalitis. But, unfortunately, gastroenteritis due to *L. monocytogenes* has been reported (Ooi and Lorber, 2005). Scallan et al. (2011) estimated the annual number of foodborne illnesses caused by 31 pathogens in the United States. Of these, there are over 3.6 million foodborne illnesses caused by pathogenic bacteria annually. Over 60 thousand of these cases are caused by Shiga toxin-producing *Escherichia coli* (STEC) O157. About a million other cases are caused by *Salmonella* spp. and over one thousand cases are caused by *L. monocytogenes*. In the

case of *L. monocytogenes*, the total number of illnesses is much fewer than for STEC O-157 or *Salmonella* spp., but the death rate is 15.9%, which is much higher than that of STEC O-157 or *Salmonella* spp.

Apple juice is a rich source of antioxidants that may defend humans from free radical damage (Lee et al., 2003). Antioxidants in apple juice, such as flavonoids, can reduce coronary heart disease and cancer (Hertog et al., 1993). Apple juice consumption is continuously increasing because of its health benefits, as well its taste and flavor (USDA, 2013). Generally, apple juice is believed to be free from the presence of foodborne pathogens because it has low pH in the range of 3.3 - 4.1 (Mattick and Moyer, 1983). But, unfortunately, there have been several outbreaks caused by apple juice (Sivapalasingam et al., 2004; Rangel et al., 2005). In 1996, in the USA, there was a large outbreak caused by *E. coli* O157:H7 in unpasteurized apple juice which resulted in 25 hospitalizations and 1 death (Cody et al., 1999). Between 1995 and 2005, in the USA, there were 5 incidents of juice-associated outbreaks caused by *Salmonella*. Due to these outbreaks, 710 people contracted illnesses (Vojdani et al., 2008). There have been no juice-

related outbreaks associated with *L. monocytogenes*, but it was identified as a potential pathogen which threatens juice safety in the final ruling published in the Federal Register (Gabriel and Nakano, 2009).

Today, pasteurization of juice by conventional heating is undesirable because juice quality deterioration occurs during thermal treatment. During high temperature-short time pasteurization (HTST), loss of flavoring agents occurred (Aguilar-Rosas et al., 2007). Therefore, many researchers are focusing on novel methods for non-thermal pasteurization of juices. Ozone is one of the most popular non-thermal pasteurization methods. In 2001, the USFDA approved gaseous and aqueous ozone treatment as an antimicrobial agent for food (Khadre et al., 2001). After this approval several studies which confirmed the microbial inactivation effect of ozone on juices were reported. Ozone showed great antimicrobial effect on orange juice, apple cider (Williams et al., 2004) and apple juice (Choi et al., 2012).

The effect of ozone is affected by pH. Patil et al. (2010b) reported that the t_{5d} of *E. coli* ATCC 25922 and NCTC 12900 (non-toxigenic strains) in pH 5.0 apple juice was much higher than in pH 3.0 juice. But there has been

no study investigating the antimicrobial effect of ozone on foodborne pathogens in apple juice of different pH levels. Commercial apple juice is not a low a_w foods, but the effect of pH on inactivation of foodborne pathogens by ozone treatment is should be confirmed because juice concentrate (usually $a_w < 0.8$) has low pH. Before I apply ozone treatment on juice concentrate, preliminary experiment is needed. Therefore, this study was undertaken to evaluate the antimicrobial effect of ozone on *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in apple juice at different pH levels. To ascertain quality changes due to ozone treatment, color values, non-enzymatic browning indices and total phenolic contents were investigated.

III-1.2. Materials and Methods

Bacterial strains and inoculum preparation Three strains of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971, ATCC 700408), and *L. monocytogenes* (ATCC 19114, ATCC 19115, ATCC 15313) were obtained from the bacterial culture collection of Seoul National University (Seoul, Korea). Stock cultures were prepared by growing strains at 37°C in Tryptic Soy Broth (TSB; Difco, BD, Sparks, MD) for 24 h, combining 0.7 ml of the stationary phase cultures with 0.3 ml of sterile 50% glycerol, and storing at -80°C in vials. Bacteria were streaked onto Tryptic Soy Agar (TSA; Difco, BD), incubated at 37°C for 24 h and stored at 4°C before use. Each strain of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was cultured in 5 ml TSB at 37°C for 24 h and centrifuged at 4000 x g for 20 min at 4°C. Cell pellets were washed three times with sterile 0.2% peptone (Bacto, Sparks, MD) water and resuspended. After this, the final cell concentration was approximately 8-9

log CFU/ml. To inoculate apple juice, three strains of each bacterium were equally combined to construct a mixed culture cocktail.

Sample preparation and inoculation Non-frozen apple juice concentrate (72 °Brix, a_w 0.75) was purchased at a local market (Chung-Book, Korea) and diluted with sterile distilled water to 18 °Brix, because apple juice usually has a soluble solids content ranging from 10 to 22 °Brix (Eisele and Drake, 2005). Soluble solids content was measured with a digital refractometer (Atago Co., Ltd.). Initial pH of 18 °Brix apple juice was 2.51 and a_w was 0.98. The pH of samples was adjusted to 3.0, 4.0 and 5.0 with 40 g/l (1 M) sodium hydroxide. During juice preparation, pH was measured with a pH meter (Mettler-Toledo, Switzerland). Then, 200 ml of each apple juice sample was dispensed into a 500 ml bottle along with 0.1 ml of antifoam B Emulsion (Sigma Aldrich, Ireland Ltd.) to prevent the overflow of juice. After that, 0.1 ml of mixed culture cocktail (*E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes)* was inoculated into the

apple juice immediately before ozone treatment. The final cell concentration was approximately 5-6 log CFU/ml.

Ozone treatment The apparatus used in this study was the same as the one used in our previous investigation (Fig. III-1) (Sung et al., 2014; Song et al., 2014a). Gaseous ozone was produced from pure oxygen with an ozone generator (Ozonetech Co., Ltd, Korea) at generation rates of 2.0-3.0 g/m^3 and a flow rate of 3.0 l/min. An ozone detector (Okitrotec Co., Japan) was used to continuously monitor the concentration of ozone gas. Pumped ozone was delivered through a tube and sparged directly into the sample with a porous tube. The apple juice samples were treated for 1, 2, 3 and 4 min in a shaking water bath at a mixing rate of 150 rpm to achieve uniform distribution. During ozone treatment the temperature was held at 22°C.

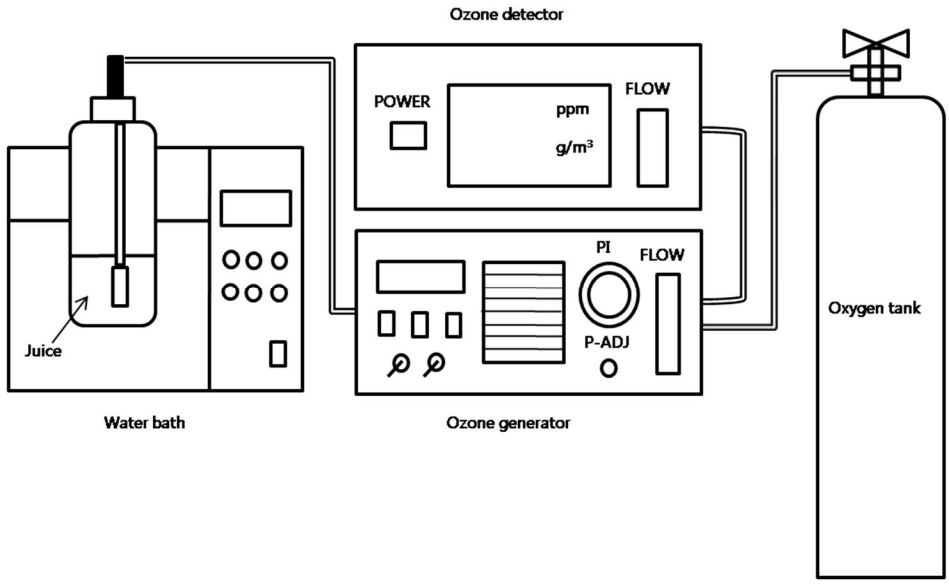

Figure III-1. Schematic diagram of the ozone treatment apparatus at Seoul National University (Seoul, Republic of Korea) (Sung et al. 2014).

Microbiological analysis After ozone treatment, 1 ml sample aliquots were transferred into test tubes containing 9 ml of D/E neutralizing broth (Difco, Becton Dickinson, Sparks, MD, USA). Each test tube was homogenized with a vortex mixer (VM-10, Daihan Scientific co., Ltd, Korea). Then, samples were 10-fold serially diluted with 0.2% sterile peptone water and 0.1 ml of diluted samples was spread-plated onto selective media. Sorbitol MacConkey Agar (SMAC; Difco), Xylose Lysine Desoxycholate Agar (XLD; Difco) and Oxford Agar Base with antimicrobial supplement (OAB; MB Cell) were used as selective media to enumerate *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Where low populations of surviving cells were anticipated, 0.25 ml aliquots of the original homogenate were pipetted onto four plates of each respective medium and spread-plated. All plates were incubated at 37°C for 24 h before enumeration.

Color measurement Color values of *L*, *a,* and *b* were used to evaluate the color changes of apple juice at the three pH levels after ozone treatment.

Color values were measured by using a Minolta colorimeter (model CR400; Minolta Co., Osaka, Japan). Non-ozone treated apple juice was used as the control. Two ml of sample was placed in the bottom half of the measurement device. The measuring head of the colorimeter was located on top of the measurement device. Color values for the control and treatments were measured after storage at 4°C for 0, 1, 3, 5 and 7 days. The parameters *L, a,* and *b* values are indicators of lightness, redness and yellowness, respectively. All measurements were performed in triplicate.

Non-enzymatic browning index measurement The non-enzymatic browning index was determined by a modification of the method described by Caminiti et al. (2012). Five ml of sample was mixed with 5 ml of 95% ethanol, centrifuged for 10 min at 8000 x g and absorbance of the supernatant was measured by a spectrophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA) at 420 nm. Non-enzymatic browning is a valuable quality indicator since it is an important chemical reaction causing quality and color changes during storage of citrus products such as

apple juice (Bharate and Bharate, 2014). The non-enzymatic browning index for the controls and treatments was measured after storage at 4°C for 0, 1, 3, 5 and 7 days. All measurements were performed in triplicate.

Total phenolic content measurement Total phenolic content was determined by colorimetric assay based on the procedure of Singleton and Rossi (1965) and modified by Torlak (2014). Briefly, 1 ml of a 10 fold diluted with distilled water juice sample was mixed with 5 ml of 0.2 N Folin–Ciocalteu reagent (Sigma-Aldrich, St. Loouis, MO, USA). After maintaining the mixture at room temperature (22°C) for 3 min, 4 ml of 7.5% sodium carbonate (Samchun Pure Chemicals Co., Ltd., Pyeongtaek, Republic of Korea) solution was added to the mixture and then shaken. After storage for 2 h at room temperature in the dark, absorbance was measured at 765 nm against a blank sample using a spectrophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA). The results were expressed as gallic acid (Sigma-Aldrich, St. Louis, MO, USA) equivalents (mg GAE/l).

Statistical analysis All experiments were duplicate-plated and replicated three times. All data were analyzed with one-way ANOVA using the Statistical Analysis System (SAS Institute, Cary, NC, USA) and Duncan`s multiple range test to determine if there were significant differences ($P < 0.05$) in mean values of pathogen reductions, Hunter color values, browning indices and total phenolic contents. Microbial counts were transformed to $\log_{10}$ values for analysis.

III-1.3. Results

Initial populations of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in inoculated apple juice were approximately 10^5-10^6 CFU/ml and the detection limit was 1.0 log CFU/ml. Ozone treatment exhibited a great effect in reducing *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* in apple juice of varying pH. Fig. III-2 through III-4 represent the populations of surviving *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes,* respectively, at each of three different pH levels (3.0, 4.0 and 5.0) of apple juice after treatment.

Fig. III-2 shows the bactericidal effect of ozone against *E. coli* O157:H7 in apple juice of pH 3.0, 4.0, and 5.0. The levels of surviving pathogens were reduced in all treated apple juice samples as treatment time increased from 0 min to 4 min. When pH 3.0 apple juice was treated with ozone (1, 2, 3 and 4 min), populations of *E. coli* O157:H7 were decreased by 0.17, 1.97, 4.10 and > 5.36 log CFU/ml, respectively. Ozone treatment for 1, 2, 3 and 4 min inactivated *E. coli* O157:H7 by 0.02, 1.69, 3.74 and 5.12 log CFU/ml in

pH 4.0 apple juice, respectively. In the case of pH 5.0 apple juice, ozone treatment for 1, 2, 3 and 4 min reduced *E. coli* O157:H7 by 0.05, 0.23, 0.68 and 1.86 log CFU/ml, respectively.

The reduction of *S.* Typhimurium in pH 3.0, 4.0, and 5.0 apple juice following ozone treatment is shown in Fig. III-3. The reduction trend was similar to that of *E. coli* O157:H7. Levels of surviving populations were decreased by 0.24, 1.01, 3.42 and > 5.23 log CFU/ml in pH 3.0 apple juice after 1, 2, 3 and 4 min ozone treatment, respectively. In pH 4.0 apple juice treated with ozone for 1, 2, 3 and 4 min, *S.* Typhimurium was decreased by 0.12, 0.88, 2.28 and 4.71 log CFU/ml, respectively. In pH 5.0 apple juice, ozone treatment for 1, 2, 3 and 4 min inactivated 0.12, 0.37, 0.81 and 1.30 log CFU/ml of this pathogen, respectively.

Fig. III-4 shows the reduction of *L. monocytogenes* by ozone treatment. It was not different from reduction trends of *E. coli* O157:H7 and *S.* Typhimurium. Ozone treatment for 1, 2, 3 and 4 min reduced this pathogen by 0.10, 0.55, 1.95 and > 4.17 log CFU/ml in pH 3.0 apple juice, respectively. In pH 4.0 apple juice treated with ozone for 1, 2, 3 and 4 min,

L. monocytogenes was decreased by 0.25, 0.99, 1.59 and 3.75 log CFU/ml respectively. In the case of pH 5.0 apple juice, ozone treatment for 1, 2, 3 and 4 min reduced *L. monocytogenes* by 0.27, 0.82, 1.30 and 1.59 log CFU/ml, respectively.

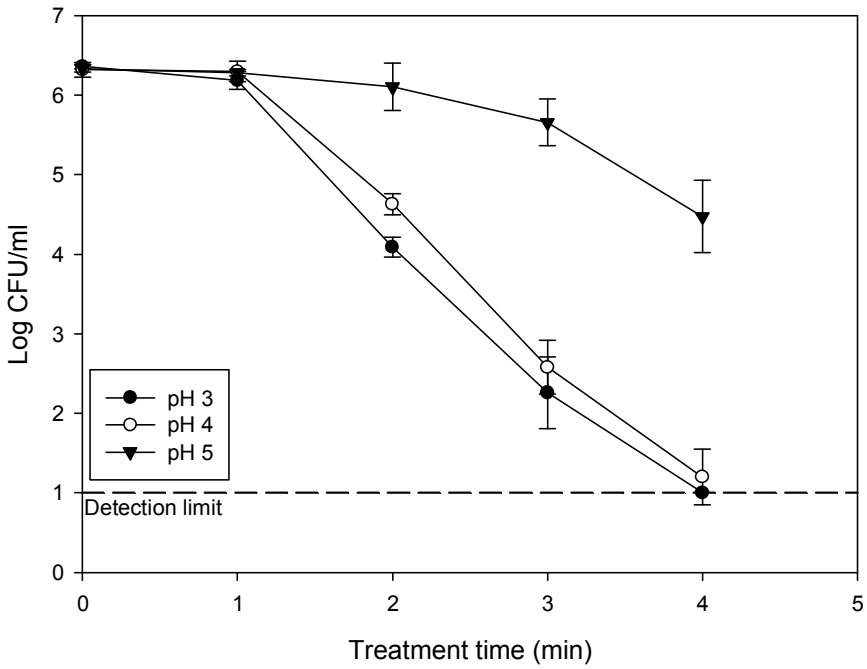

Figure III-2. Inactivation of *E. coli* O157:H7 in apple juice at different pH levels treated with gaseous ozone treatment. ● pH 3.0 apple juice; ○ pH 4.0 apple juice; ▼pH 5.0 apple juice.

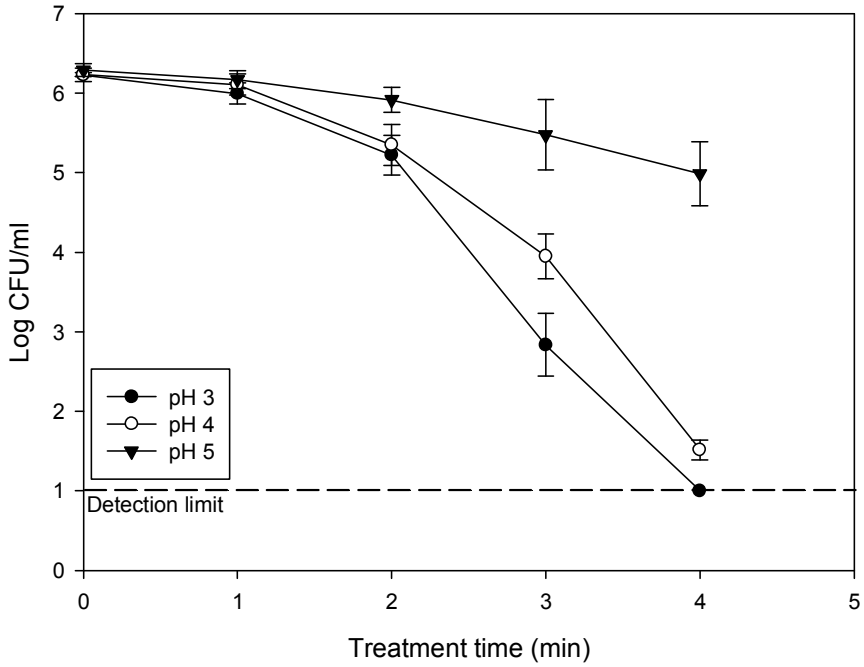

Figure III-3. Inactivation of *S.* Typhimurium in apple juice at different pH levels treated with gaseous ozone treatment. ● pH 3.0 apple juice; ○ pH 4.0 apple juice; ▼pH 5.0 apple juice.

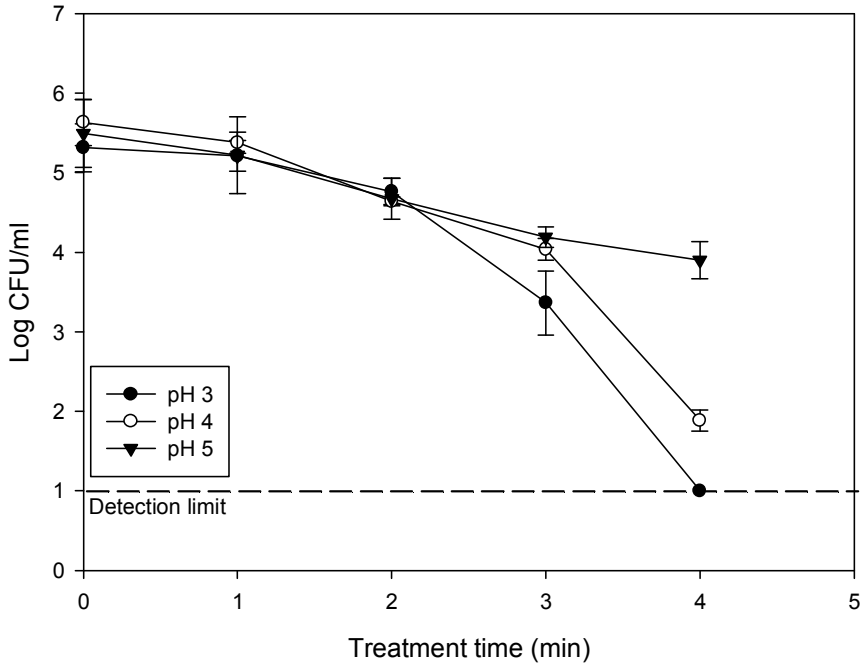

Figure III-4. Inactivation of *L. monocytogenes* in apple juice at different pH levels treated with gaseous ozone treatment. ● pH 3.0 apple juice; ○ pH 4.0 apple juice; ▼pH 5.0 apple juice.

When we treated apple juice for 1 min there were no significant differences in reduction between any of the pathogens. In pH 3.0 apple juice, *E. coli* O157:H7 was reduced faster than the other two pathogens, but there were no significant differences between *E. coli* O157:H7 and *S.* Typhimurium when apple juice was treated with ozone for 3 or 4 min. *L. monocytogenes* populations decreased slower than the other two pathogens but this pathogen was reduced to under the detection limit after 4 min ozone treatment. This trend was also observed in the case of pH 4.0 apple juice. But, in pH 5.0 apple juice, there were no significant differences in the rate of reduction between any of the three pathogens, except that samples treated with ozone for 2 min showed greater reduction of *L. monocytogenes*.

The Hunter color values of apple juice treated with ozone for 4 min and non-treated apple juice are shown in Table III-1. In all ozone treated apple juice samples of pH 3.0, 4.0, and 5.0, *L* and *a* values were not significantly different ($P > 0.05$) from those of the control. But *b* values of ozone treated samples were significantly lower than those of non-treated samples. During storage, no significant changes were observed.

TABLE III-1 The Hunter's color *L* (lightness), *a* (redness), and *b* (yellowness) values of gaseous ozone treated (4 min) and non-treated apple juices at different pH levels during storage at 4°C.

Parameter	Treatment	Day 0	Day 1	Day 3	Day 5	Day 7
L	pH 3	24.47 ± 0.16[a] A	24.60 ± 0.23 A	24.47 ± 0.28 A	24.52 ± 0.07 A	24.41 ± 0.26 A
	pH 3, ozone	24.64 ± 0.33 A	24.66 ± 0.27 A	24.56 ± 0.15 A	24.62 ± 0.12 A	24.67 ± 0.12 A
	pH 4	24.49 ± 0.08 A	24.59 ± 0.05 A	24.47 ± 0.30 A	24.55 ± 0.06 A	24.52 ± 0.24 A
	pH 4, ozone	24.71 ± 0.24 A	24.81 ± 0.15 A	24.54 ± 0.18 A	24.76 ± 0.21 A	24.60 ± 0.17 A
	pH 5	24.85 ± 0.24 A	24.61 ± 0.12 A	24.54 ± 0.07 A	24.66 ± 0.20 A	24.52 ± 0.28 A
	pH 5, ozone	24.80 ± 0.27 A	24.84 ± 0.08 A	24.75 ± 0.09 A	24.75 ± 0.10 A	24.83 ± 0.24 A
a	pH 3	0.34 ± 0.02 A	0.34 ± 0.00 A	0.35 ± 0.01 A	0.33 ± 0.01 A	0.34 ± 0.03 A
	pH 3, ozone	0.36 ± 0.01 A	0.34 ± 0.01 A	0.37 ± 0.05 A	0.34 ± 0.04 A	0.34 ± 0.01 A
	pH 4	0.31 ± 0.05 A	0.35 ± 0.03 A	0.35 ± 0.01 A	0.34 ± 0.04 A	0.34 ± 0.02 A
	pH 4, ozone	0.32 ± 0.03 A	0.33 ± 0.00 A	0.35 ± 0.03 A	0.31 ± 0.01 A	0.33 ± 0.02 A
	pH 5	0.32 ± 0.04 A	0.35 ± 0.04 A	0.34 ± 0.01 A	0.31 ± 0.04 A	0.31 ± 0.03 A
	pH 5, ozone	0.35 ± 0.04 A	0.34 ± 0.01 A	0.34 ± 0.05 A	0.31 ± 0.03 A	0.32 ± 0.02 A
b	pH 3	4.56 ± 0.01 A	4.49 ± 0.07 A	4.50 ± 0.06 A	4.58 ± 0.11 A	4.57 ± 0.11 A
	pH 3, ozone	4.34 ± 0.08 B	4.28 ± 0.04 B	4.32 ± 0.11 B	4.38 ± 0.07 B	4.32 ± 0.05 B
	pH 4	4.52 ± 0.02 A	4.48 ± 0.06 A	4.45 ± 0.14 A	4.54 ± 0.09 A	4.52 ± 0.08 A
	pH 4, ozone	4.40 ± 0.09 B	4.35 ± 0.05 B	4.35 ± 0.05 A	4.38 ± 0.03 B	4.42 ± 0.03 A
	pH 5	4.57 ± 0.05 A	4.52 ± 0.07 A	4.51 ± 0.05 A	4.56 ± 0.11 A	4.58 ± 0.07 A
	pH 5, ozone	4.33 ± 0.03 B	4.28 ± 0.09 B	4.29 ± 0.04 B	4.31 ± 0.02 B	4.30 ± 0.01 B

[a] Mean values ± standard deviation. Means followed by the same letter within a column per parameter and pH are not significantly different. There were no significant differences within a row ($P < 0.05$).

Browning indices of ozone treated and non-treated apple juice are shown in Table III-2. Ozone treated apple juice showed significantly lower browning indices than non-treated apple juice. During storage, no significant changes of non-treated samples were observed, except in pH 5.0 apple juice. Ozone and non-ozone treated pH 5.0 apple juice showed a lower browning index after storage.

TABLE III-2 Browning indices of gaseous ozone treated (4 min) and non-treated apple juices at different pH levels during storage at 4°C.

	Day 0	Day 1	Day 3	Day 5	Day 7
pH 3 non treated	0.274 ± 0.016[a] Aa	0.283 ± 0.022 Aa	0.287 ± 0.010 Aa	0.284 ± 0.003 Aa	0.285 ± 0.028 Aa
pH 3 ozone treated	0.213 ± 0.011 Ba	0.224 ± 0.015 Ba	0.229 ± 0.009 Ba	0.237 ± 0.008 Ba	0.230 ± 0.021 Ba
pH 4 non treated	0.243 ± 0.011 Aa	0.241 ± 0.015 Aa	0.235 ± 0.008 Aa	0.232 ± 0.003 Aa	0.232 ± 0.012 Aa
pH 4 ozone treated	0.206 ± 0.008 Ba	0.195 ± 0.012 Ba	0.192 ± 0.005 Ba	0.196 ± 0.003 Ba	0.192 ± 0.007 Ba
pH 5 non treated	0.258 ± 0.008 Aa	0.226 ± 0.006 Ab	0.236 ± 0.005 Ab	0.230 ± 0.002 Ab	0.229 ± 0.006 Ab
pH 5 ozone treated	0.222 ± 0.003 Ba	0.195 ± 0.008 Bb	0.199 ± 0.004 Bb	0.194 ± 0.005 Bb	0.191 ± 0.002 Bb

[a] Mean values ± standard deviation. Means followed by the same uppercase letters within a column per pH and by the same lowercase letters within a row are not significantly different ($P< 0.05$).

Total phenolic contents of apple juice treated and non-treated with ozone for 4 min are shown in Table III-3. In all ozone treated apple juice samples of different pH, total phenolic contents were not significantly different ($P > 0.05$) from those of the control.

TABLE III-3 Total phenolic content of gaseous ozone treated (4 min) and non-treated apple juices at different pH levels.

pH	Total phenolic content (mg GAE/l)	
	Non-treated sample	Ozone treated sample
3	144.1 ± 7.8[a]	141.9 ± 11.9
4	148.6 ± 9.7	139.8 ± 1.7
5	140.7 ± 8.7	140.7 ± 9.5

[a] Mean values ± standard deviation. Mean values in same column and row did not differ significantly ($P< 0.05$).

III-1.4. Discussion

Traditionally, apple juice has been pasteurized by conventional heating. HTST pasteurization is the most common pasteurization system used by the juice industry which uses a temperature range of 76.6 to 87.7°C and a holding time range of 20 to 30 s. But as we mentioned previously, HTST pasteurization leads to quality deterioration of apple juice (Aguilar-Rosas et al., 2007). Therefore, many researchers have focused on non-thermal treatments to inactivate microorganisms in apple juice, such as pulsed light treatment (Sauer and Moraru, 2009) and ultraviolet light (Caminiti et al., 2012).

Ozone is an allotropic form of oxygen which has great oxidation potential and can act as a powerful antimicrobial agent (Guzel-Seydim et al., 2004). It has an oxidation potential of 2.07 V in alkaline solution which is higher than that of chlorine (1.36 V) and also does not produce any harmful byproducts, such as trihalomethanes (THMs) which can be produced by chlorine (Glaze, 1987; Sung et al., 2014). Excess ozone rapidly decomposes

into diatomic oxygen (O_2) (Khadre et al., 2001). Ozone reduces microorganisms by attacking various cellular elements such as lipoprotein and lipopolysaccharide layers in gram negative bacteria and double bonds of unsaturated lipids in the cell envelope, resulting in cell lysis (Scott and Lesher, 1963; Murray et al., 1965).

Patil et al. (2010b) reported on the inactivation effect of non-pathogenic *E. coli* by ozone on apple juice at different pH levels. The time required for 5 log reduction of *E. coli* increased as pH increased from 3.0 to 5.0. This supports the results of the present study which show that ozone treatment of low pH apple juice shows great antimicrobial activity. When pathogens are inoculated into pH 3.0 apple juice and held for 4 min, populations of pathogens were not significantly changed (data not shown). But ozone treatment for 4min reduced populations of the three pathogens to under the detection limit in pH 3.0 apple juice, whereas in pH 5.0 juice, pathogens were reduced by 1.30 to 1.86 log CFU/ml. Low pH and ozone treatment showed a synergistic antimicrobial effect. These results can be explained in two aspects. First, ozone reacts in two ways with organic compounds: direct

reaction of the ozone molecule with organic compounds or decomposition of the ozone molecule to produce radicals which can then react with these compounds (Staehelin and Hoigné, 1985). Ozone is more stable at low than at high pH (Khadre et al., 2001), which means direct reaction with ozone molecules accounts for the majority of pathogen inactivation in low pH apple juice. Finch et al. (1992) and Labatiuk et al. (1994) also reported that direct reaction with molecular ozone is more effective for inactivation of microorganisms than reaction with radicals arising from decomposition of ozone molecules. Second, the combination effect of ozone with low pH. Low pH produces antimicrobial and growth inhibition effects. These effects occur due to disruption of the outer membrane of gram negative microorganisms (Paul Ross et al., 2002). Alakomi et al. (2000) reported that NPN (1-*N*-phenylnaphthylamine) uptake by *E. coli* and *Salmonella enterica* serovar Typhimurium at low pH (pH 3.6 and 4.0 adjusted with lactic or hydrochloric acid) was much larger than that at neutral pH (pH 7.2). Uptake of NPN through the bacterial membrane is an indicator of damage in the outer membrane of gram negative bacteria. Low pH disrupts the outer

membrane of gram negative bacteria resulting in its permeabilization which allows passage of ozone through the cell membrane and enhances the antimicrobial effect of ozone. In the case of gram positive bacteria, weak acids produce a potential antimicrobial effect through permeabilization of the cell membrane and diffusion of undissociated forms of weak acids through the cell membrane. Weak acids then dissociate and release protons which result in lowering the cytoplasmic pH. By contrast, cytoplasm of cells with intact membranes has higher pH than that of general growth media (Cotter and Hill, 2003). Malic acid, a weak organic acid, is the predominant acid found in apple juice. When we treated gram positive bacteria in apple juice with ozone, the cell membrane was permeabilized due to ozone damage which enhanced entry of malic acid into the cytoplasm resulting in an enhanced antimicrobial effect.

During oxidative treatment involving reactive oxygen species including ozone, injury of foodborne pathogens can occur (Stephens et al., 2000; Thanomsub et al., 2002). Injured cells can not form colonies on selective agar because selective agents such as antibiotics, dyes, or bile salts interfere

with growth of injured microorganisms (Prentice and Clegg, 1974). For these reasons, the inactivation effect of gaseous ozone treatment on *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* could be overestimated. But there are some studies which show that during storage following control interventions, injured pathogens in fruit juice were reduced continuously. Jordan et al. (2001) reported that high hydrostatic pressure of 500 MPa for 5 min achieved *E. coli* O157 C9490 reductions of about 1 - 2 log in orange juice. But following storage of pressure-treated orange juice at 4°C for 24 h and at 25°C for 3 h, *E. coli* O157 C9490 was reduced by 4.4 and > 7 log CFU/ml, respectively. *L. monocytogenes* showed a similar trend to that of *E. coli* O157. Also, García et al. (2005) reported that during storage at 4°C after pulsed electric field treatment, *E. coli* O157:H7 in apple juice reduced consistently. They stated that pulsed electric field treatment of *E. coli* O157:H7 in apple juice produced injured cells which became more sensitive to subsequent storage under refrigeration. Ozone can also injure pathogens, but injured cells are not able to adapt to the storage environment of apple juice (low pH and low temperature).

Ozone can be used as a decolorizing agent because it has a great oxidation potential. Ozone breaks double bonds between carbon (-C=C-), nitrogen (-N=N-) and heterocyclic, aromatic rings in foods (Strickland and Perkins, 1995). Torres et al. (2011) reported that ozone treatment (4.8% w/w) for 10 min in apple juice resulted in increased *L* and *b* values and decreased *a* value. In our study, there were no significant changes of *L* and *a* values. But the *b* value of apple juice was decreased after ozone treatment for 4 min. There are studies which confirmed that ozone treatment (3 to 4 min) decreased the *b* value of strawberry juice, orange juice and grape juice (Tiwari et al., 2008; Tiwari et al., 2009b; Tiwari et al., 2009c). However, in this study, changes in *b* value were very small and those could not be detected visually. During 7 days storage at 4°C, no significant changes were observed, which means that color of apple juice was affected by ozone during treatment but not affected after treatment.

Browning is one of the main quality deteriorations of citrus juices, and can occur during thermal treatment and storage. Toribio and Lozano (1986) reported that conventional heating increased browning of apple juice of

varying solids content. Leizerson and Shimoni (2005) reported that ohmic heating induced non-enzymatic browning of orange juice during storage at 4°C. In the case of apple juice, non-enzymatic browning by the Maillard reaction is the main cause of browning (Toribio and Lozano, 1984). The Maillard reaction takes place between reducing sugars and amino acids and ultimately yields melanoidin which is manifested by brown color (Baltes, 1982; Toribio and Lozano, 1984). In this study, we can confirm that ozone reduced the browning index in apple juice and did not stimulate browning during storage. During storage there was no significant difference observed except in pH 5.0 apple juice which showed a reduced browning index. Reducing browning indices were also observed during other non-thermal treatments of apple juice. Caminiti et al. (2012) reported that during UV treatment browning indices were reduced, and a reduced browning index during gamma irradiation treatment was also reported (Fan and Thayer, 2002).

Total phenolic content is one of the main factors responsible for health benefit of consuming apple juice (Kahle et al., 2005). But several

researchers reported that during ozone treatment of apple juice total phenolic content was reduced. Torlak (2014) reported that ozone treatment (2.8 and 5.3 mg/l) of apple juice at 4 and 22°C for 40 min produced significant reductions of total phenolic content. Torres et al. (2011) reported that total phenolic content of fresh apple juice was 63.8 mg GAE/ml but that of ozone treated (4.8% for 10 min) apple juice was 32.1 mg GAE/ml. In our study, there were no significant differences of total phenolic contents between ozone treated and non-treated samples. Changes in total phenolic content of apple juice during ozone treatment can be affected by various control parameters such as ozone concentration and treatment time. It is noteworthy that in the current study treatment time was shorter and ozone concentration was lower than that of the cited investigations.

Our research showed that gaseous ozone can be used as a pasteurization intervention for apple juice at different pH levels. Ozone treatment for 4 min resulted in 1.30 to 1.86 log reductions of three pathogens in pH 5.0 apple juice and reduced levels of three pathogens to under the detection limit in pH 3.0 apple juice. During ozone treatment, *b* values were decreased but

could not be detected with the unaided eye. Ozone treatment reduced browning of apple juice and did not result in browning during storage at 4°C. Also, ozone treatment of apple juice of different pH levels did not affect the total phenolic content. In this study, we found there were large significant differences in surviving pathogen populations between pH 3.0 and 5.0 apple juice, but no large differences between pH 3.0 and 4.0 apple juice. Therefore, further investigations are needed to confirm the exact pH which shows the synergistic effect with ozone and also elucidates the mechanism of the combination effect of ozone and low pH.

III-2. Inactivation of foodborne pathogens in black and red pepper by vacuumed hydrogen peroxide vapor

III-2.1. Introduction

Enterohaemorrhagic *Escherichia coli* O157:H7 causes hemorrhagic colitis (bloody diarrhea), non-bloody diarrhea and the very serious hemolytic uremic syndrome (Kaper et al., 2004). In the USA, 53 outbreaks of *E. coli* O157:H7 infections were reported between 2009 and 2010 and 34 multistate outbreaks of shiga-toxin producing *E. coli* infections were reported between 2010 to 2014 (CDC 2013, 2015). *S. enterica* serovar Typhimurium is the most commonly isolated *Salmonella* serotype and causes a self-limiting gastroenteritis that may lead to systemic disease (Hulme et al., 2017). Between 2009 and 2010, there were 27 outbreaks in the USA caused by *S.* Typhimurium (CDC, 2013). Scallan et al. (2011) estimated there are over 9 million foodborne illnesses in United States annually, about a million cases of them are caused by *Salmonella* spp. and 63,153 cases of them are caused by Shiga toxin-producing *Escherichia coli* (STEC) O157.

Black and red pepper are universally-recognized spices that have been in

use for a long time. These peppers usually contain large populations of micro flora. Oularbi and Masouri (1996) reported that microbial loads of black and red pepper were 10^7-10^8 log CFU/g and Buckenhüskes and Rendlen (2004) reported that microbial counts of black pepper and red pepper were 5 x 10^5 – 1 x 10^7 and 1 x 10^5 – 5 x 10^5 CFU/g, respectively. Moreover, pathogen-contaminated spices are wide spread as I mentioned in Chapter I.

A number of methods to reduce foodborne pathogens and microorganisms in black and red pepper have been developed, such as ozone treatment and steaming. Zhao and Cranston (1995) reported that ozone treatment (40 mg/min for 60 min) of ground black pepper produced more than 3 log CFU/g reductions of *E. coli* and *Salmonella*. Akbas and Ozdemir (2008) also used ozone for reducing pathogens in red pepper but 1.0 ppm of ozone treatment for 6 h reduced *E. coli* by only 2 log CFU/g. However, ozone treatment is not suitable for pasteurization of black and red pepper because of the longer treatment time. Duncan et al. (2017) reported that vacuum assisted steam (82.22°C, 7.5 psia) treatment of peppercorns

which produced 5 log CFU/g reductions of *Salmonella* (Newkirk, 2016) affected the color of black pepper. Waje et al. (2008) and Rico et al. (2010) also used steam treatment (at 1020 mbar 100°C for 16 min) on black and red pepper, respectively. But steam treatment yielded less than 3 log CFU/g and 1 log CFU/g reductions of total microorganisms in black and red pepper, respectively, and affected color values of black and red pepper. These results show that steam treatment negatively affects the color of black and red pepper causing significant degradation of color values. Therefore, new technologies are needed for decontamination of black and red pepper while maintaining product quality.

Hydrogen peroxide has been widely investigated for inactivating microorganisms in foods and is a GRAS (generally recognized as safe) compound. Hydrogen peroxide forms the hydroxyl radical (•OH) which oxidizes cell membranes (McDonnell and Russell, 1999). Svoboda et al. (2016) reported that 5% hydrogen peroxide treatment for 2 min reduced *E. coli* O157:H7 and *Salmonella* on the surface of melons by about 1.5 log CFU/g. This result shows that hydrogen peroxide usually needs to be used

in a combination treatment because processing with hydrogen peroxide alone does not effectively reduce pathogens in food. Shamila-Syuhada et al. (2016) reported that a combination treatment of hydrogen peroxide and ultra sound effectively reduced pathogens in milk. Hong and Kang (2016) reported that the combination of hydrogen peroxide and dry heat is effective for seed pasteurization. Also, the combination treatment of steam and hydrogen peroxide effectively eliminated pathogen biofilms on stainless steel surfaces (Ban and Kang, 2016).

Back et al. (2014) reported that 10% hydrogen peroxide vapor (HPV) for 10 min reduces levels of *E. coli* O157:H7, *S.* Typhimurium and *Listeria monocytogenes* on lettuce by about 3 log CFU/g without inducing quality deterioration. Recently, many medical sterilizers have been developed which use hydrogen peroxide vapor as a sterilant in vacuum chambers. But, to date, there have been no studies documenting inactivation of pathogens in black and red pepper by using vacuumed hydrogen peroxide vapor. Therefore, the objectives of this study were to evaluate efficacy of vacuumed hydrogen peroxide vapor to inactivate *E. coli* O157:H7 and *S.*

Typhimurium in black and red pepper and confirm quality changes during vacuumed hydrogen peroxide vapor processing by color values measurement.

III-2.2. Materials and Methods

Bacterial strains and cell suspension Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890) and *S.* Typhimurium (ATCC 19586, ATCC 43174, ATCC 700408) were used. Each strain was obtained from the bacteria culture collection of Seoul National University (Seoul, Korea) for this study. Stock cultures were prepared by mixing 0.7 ml of a tryptic soy broth (TSB; Difco, BD, Sparks, MD) 24 h, 37°C culture with 0.3 ml of sterile 50% glycerol and then storing at –80°C. Working cultures were streaked onto tryptic soy agar (TSA; Difco, BD), incubated at 37°C for 24 h and stored at 4°C. Each strain of *E. coli* O157:H7 and *S.* Typhimurium was cultured in 5 ml TSB at 37°C for 24 h, harvested by centrifugation at 4000 × *g* for 20 min at 4°C and washed three times with peptone water (PW; Difco, Sparks, MD). The final pellets were resuspended in 0.2% PW, corresponding to approximately 10^8-10^9 CFU/ml. Subsequently, suspended pellets of each strain of the two pathogen species were combined to produce culture cocktails.

Sample preparation, inoculation Whole black pepper (*Piper nigrum*) and dried red pepper (*Capsicum annuum*) used for this study were purchased at a local grocery store (Seoul, Korea). For inoculation, 3.0 ml of culture cocktail was applied to 80 g samples inside sterile stomacher bags (Labplas Inc., Sainte-Julie, Quebec, Canada). The inoculated samples were mixed with hand massaging for 1 min to ensure even distribution of the pathogens. Ten g samples were placed in petri plates and dried for 1 h in a biosafety hood samples (a_w 0.35). Uniform distribution of inoculum was confirmed by similar CFU counts (10^5-10^6 CFU/g) on selective agar that were obtained from 1 g of inoculated pepper taken from random locations after the drying step.

Vacuumed hydrogen peroxide vapor treatment Vacuumed hydrogen peroxide vapor treatment was performed using a STERLINK® prototype (Plasmapp Co., Ltd., Dajeon, Republic of Korea, Fig. III-5), a newly developed apparatus for vacuum sterilization processing. This system consisted of three parts; controller, treatment chamber and vacuum pump.

The controller was used to check the chamber surface and injection port temperatures which were heated to 55°C and 220°C, respectively, and to control the chamber, needle and venting valves. The treatment chamber contained a small needle which was used for dispersion of HPV and an injection port for injection of hydrogen peroxide. The vacuum pump was used to reduce pressure inside the treatment chamber. Before processing, 3 min of preheating time was conducted for the chamber and injection port temperatures to reach 55 and 220°C, respectively. Black and red pepper samples in petri dishes were placed on the 2 cm high plastic rack (as shown in Fig. III-1) to avoid heat from the chamber surface and the cover was closed. Processing consisted of 3 steps: first pumping, diffusion and second pumping. Before the first pumping step, chamber and needle valves were closed and the venting valve was opened. The first pumping process progressed until atmospheric pressure inside the chamber was less than 1.5 torr. When chamber pressure dropped below 1.5 torr, the diffusion step commenced. Chamber and needle valves were opened and hydrogen peroxide (0.5 ml of 10, 20, 30, 40 and 50%) was injected through the

injection port by syringe. Injected hydrogen peroxide was vaporized by heating to 220°C and dispersed into the chamber. After injection, black and red pepper were exposed to HPV for 1 min. After 1 min of diffusion, the second pumping step was initiated. Chamber and needle valves were closed. This pumping step was done to remove HPV from the treatment chamber until the pressure fell below 1.5 torr. Then, the vacuum pump was turned off and the venting valve was opened. After the chamber reached atmospheric pressure, the chamber cover was opened and the samples were collected.

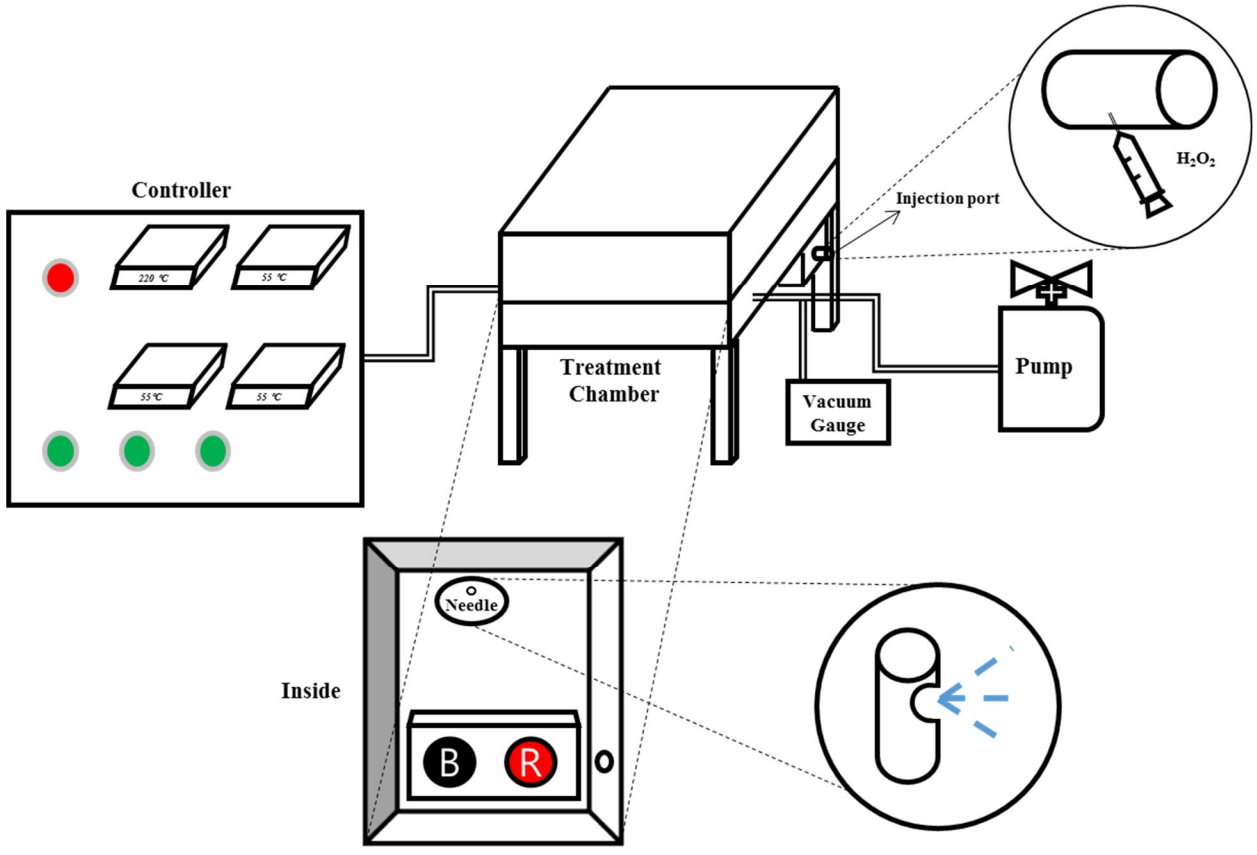

Figure III-5. Schematic diagram of the STERLINK® sterilization system at Seoul National University (Seoul, Republic of Korea).

Bacterial enumeration Collected samples (10 g) were diluted with 90 ml of 0.2% PW in a sterile stomacher bag and homogenized for 2 min in a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1 ml aliquots of homogenized samples were tenfold serially diluted in 9 ml of 0.2% PW, and 0.1 ml of sample or diluent was spread-plated onto each selective medium. Sorbitol MacConkey agar (Difco) and Xylose Lysine Desoxycholate agar (Difco) were used as selective media for the enumeration of *E. coli* O157:H7 and *S.* Typhimurium, respectively. Where low populations of surviving cells were anticipated, 1 ml aliquots of the original homogenate were equally distributed onto four plates of each respective medium and spread-plated. All plates were incubated at 37°C for 24 h and colonies were counted. Uninoculated black and red pepper samples were also processed as described above to detect any possible background contamination with *E. coli* O157:H7 or generic *Salmonella*.

Color measurement Color values of L^*, a^*, and b^* were used to quantify color attributes of samples. CIE color values (L^*, a^*, b^*) were measured

using a Minolta colorimeter (model CR300, Minolta Co., Osaka, Japan). Two g of sample was put into the bottom half of the measurement device. The measuring head of the colorimeter was placed on top of the measurement device. Color was measured from three random locations. L^*, a^*, and b^* values indicate color lightness, redness, and yellowness of the sample, respectively.

Statistical analysis All data were analyzed with one-way ANOVA using the Statistical Analysis System (SAS Institute, Cary, NC, USA) and Duncan`s multiple range test to determine if there were significant differences ($P < 0.05$) in mean values of microorganism populations. Microbial counts were transformed to log values for analysis. One log was used for calculations in the case of populations below the detection limit.

III-2.3. Results

Initial populations of *E. coli* O157:H7 and *S.* Typhimurium in inoculated black and red pepper were approximately 10^6-10^7 CFU/g. In uninoculated samples, populations of the pathogens were below the detection limit (1.0 log CFU/g). Vacuumed hydrogen peroxide vapor greatly reduced levels of *E. coli* O157:H7 and *S.* Typhimurium in black and red pepper.

The inactivation effect of vacuumed hydrogen peroxide vapor (10, 20, 30, 40 and 50%) on *E. coli* O157:H7 and *S.* Typhimurium in whole black pepper are shown in Fig. III-6. Reduction of pathogens increased with increasing vacuumed hydrogen peroxide vapor concentration from 10 to 50%. The initial population of *E. coli* O157:H7 in black pepper was 6.34 log CFU/g. After 50% vacuumed hydrogen peroxide vapor treatment, the population of this pathogen decreased to under the detection limit (1.0 log CFU/g). In 10, 20, 30 and 40% vacuumed hydrogen peroxide vapor treated samples, reductions of *E. coli* O157:H7 were 2.19, 2.78, 3.03, 3.39 and 4.30 log CFU/g, respectively. The initial population of *S.* Typhimurium in whole

black pepper was 6.02 log CFU/g. After 50% vacuumed hydrogen peroxide vapor treatment, cell numbers decreased to 1.50 log CFU/g. In 10, 20, 30 and 40% vacuumed hydrogen peroxide vapor treated samples, reductions of *S.* Typhimurium were 2.16, 2.54, 3.09, 3.49 and 4.03 log CFU/g, respectively.

Fig. III-7 shows the bactericidal effect of vacuumed hydrogen peroxide vapor (10, 20, 30, 40 and 50%) against *E. coli* O157:H7 and *S.* Typhimurium in red pepper. The populations of surviving pathogens decreased in all samples as vacuumed hydrogen peroxide vapor concentration increased from 10 to 50%. The initial population of *E. coli* O157:H7 in red pepper was 6.49 log CFU/g. After 50% vacuumed hydrogen peroxide vapor treatment levels of *E. coli* O157:H7 were reduced by 3.01 log CFU/g. In 10, 20, 30 and 40% vacuumed hydrogen peroxide vapor treated samples, reductions of *E. coli* O157:H7 were 0.99, 1.29, 1.98, 2.28 and 2.78 log CFU/g, respectively. The initial population of *S.* Typhimurium in red pepper was 6.26 log CFU/g. After 50% vacuumed hydrogen peroxide vapor treatment, the *S.* Typhimurium cell count was reduced by 2.36 log

CFU/g. In 10, 20, 30 and 40% vacuumed hydrogen peroxide vapor treated samples, reductions of *S.* Typhimurium were 1.18, 1.54, 1.82, 2.02 and 2.25 log CFU/g, respectively.

The CIE color values of black and red pepper after vacuumed hydrogen peroxide vapor treatment are shown in Table III-4 and III-5, respectively. L^*, a^*, and b^* values of vacuumed hydrogen peroxide vapor treated samples were not significantly different from those of controls ($P > 0.05$).

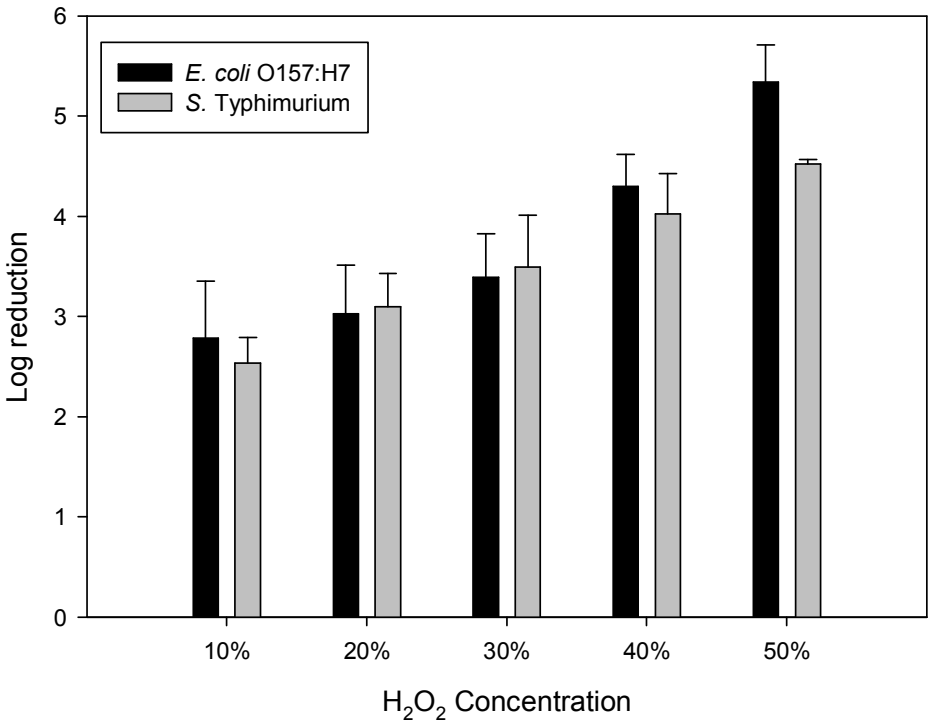

Figure III-6. Inactivation of *E. coli* O157:H7 and *S.* Typhimurium in black pepper treated with different concentrations of vacuumed hydrogen peroxide vapor.

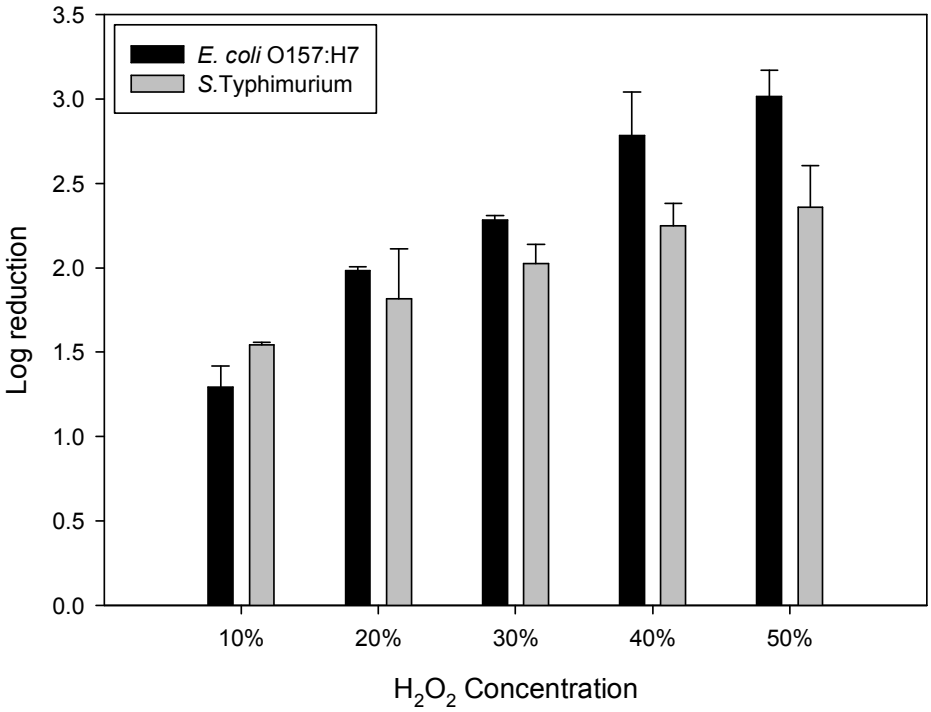

Figure III-7. Inactivation of *E. coli* O157:H7 and *S.* Typhimurium in red pepper treated with different concentrations of vacuumed hydrogen peroxide vapor.

Table III-4. CIE color values of black pepper treated with H_2O_2 vapor using the STERLINK® prototype.

Parameter	H_2O_2 Concentration					
	Control	10%	20%	30%	40%	50%
L*	20.72 ± 2.62[a]	21.25 ± 3.40	21.98 ± 2.19	20.49 ± 1.81	21.15 ± 1.71	19.78 ± 0.89
a*	3.67 ± 0.92	2.71 ± 0.98	3.35 ± 1.64	3.15 ± 0.93	3.69 ± 0.89	3.76 ± 0.68
b*	6.71 ± 3.68	4.45 ± 2.97	5.56 ± 4.06	5.55 ± 2.50	6.92 ± 1.64	6.79 ± 1.33

[a] Mean of three replications ± standard deviation. Mean values in same parameter did not significantly different ($P > 0.05$).

Table III-5. CIE color values of red pepper treated with H_2O_2 vapor using the STERLINK® prototype.

Parameter	H_2O_2 Concentration					
	Control	10%	20%	30%	40%	50%
L*	23.20 ± 1.01[a]	22.59 ± 0.82	23.28 ± 1.43	22.44 ± 1.27	22.77 ± 0.34	22.14 ± 0.79
a*	25.89 ± 1.10	25.53 ± 0.74	25.78 ± 1.56	24.02 ± 1.50	24.45 ± 0.47	23.61 ± 2.50
b*	23.15 ± 1.11	23.76 ± 1.32	23.90 ± 1.39	22.10 ± 1.46	23.00 ± 0.88	21.64 ± 3.13

[a] Mean of three replications ± standard deviation. Mean values in same parameter did not significantly different ($P > 0.05$).

III-2.4. Discussion

A number of studies have reported on the heavy microbial load of black and red pepper and detection of pathogens from those peppers (Oularbi and Mansouri 1996; Buckenhüskes and Rendlen, 2004; Choo et al., 2007). But the low water activity of these two types of peppers resulted in challenges to pasteurization by conventional thermal treatment. Thus many researchers have focused on non-thermal treatment to inactivate pathogens in peppers. Cold plasma is one of those non-thermal treatments. But usually, cold plasma treatment was not effective for pasteurization of peppers. Kim et al. (2014) reported that cold plasma treatment with a variety of gases did not reduce *B. cereus* in red pepper powder. Hertwig et al. (2015a) used remote plasma treatment to reduce total mesophilic aerobic counts. This treatment for 90 min resulted in about 4 and 3 log reductions of total mesophilic aerobic bacteria in black and red pepper, respectively. And direct plasma treatment with a radio frequency plasma jet for 15 min reduced populations of *Salmonella* in whole black peppercorns by 2-3 log CFU/g (Hertwig et al.,

2015b). However, there have been several studies which confirmed that gamma irradiation effectively reduced microorganisms in black and red peppers. Rico et al. (2010) and Waje et al. (2008) used gamma irradiation to reduce microbial loads of red pepper and black pepper, respectively. They used 10 kGy of gamma irradiation which resulted in about 5 and 4 log reductions of total plate count in red and black pepper, respectively. And Song et al. (2014b) also reported that gamma irradiation effectively reduced *E. coli* O157:H7 and *S.* Typhimurium in black and red pepper. But gamma irradiation has a big disadvantage with regard to consumer perception. These reasons have led researchers to use other interventions in combination to treat peppers. Cheon et al. (2015) reported on the effect of UV-C and mild heat combination treatment for inactivation of foodborne pathogens in red pepper. This combination treatment for 10 min (UV dose: 40.8kJ/m^2 with 65°C heating) reduced *E. coli* O157:H7 and *S.* Typhimurium by 2.88 and 3.05 log CFU/g, respectively. Also, Ha and Kang (2013) reported that the combination treatment of NIR and UV-C for 5 min reduced *Salmonella*

Typhimurium and *E. coli* O157:H7 by 3.02 and 2.55 log CFU/g, respectively.

HPV is usually used as a sterilization method for medical devices (Rutalta and Weber, 2016). There are many studies which confirm the effectiveness of HPV or the gaseous phase of hydrogen peroxide on inactivation of microorganisms. HPV effectively reduced *Bacillus anthracis* spores (Wood et al., 2016), *Clostridium difficile* endospores (Malik et al., 2016), two surrogates of human norovirus, murine norovirus and feline calicivirus (Zonta et al., 2016) and even prions (Fichet et al., 2007). But using HPV for food pasteurization has been rarely studied. Simmons et al. (1997) used HPV to reduce microbial populations of prunes and Back et al. (2014) used HPV for inactivating foodborne pathogens on fresh produce. In the present study, I confirmed that vacuumed hydrogen peroxide vapor effectively reduced pathogens in black and red pepper. But there was a significant difference ($P < 0.05$) between pathogen inactivation in black pepper versus red pepper. In 50% vacuumed hydrogen peroxide vapor treated samples, *E. coli* O157:H7 was reduced by > 5.34 and 3.01 log

CFU/g in black pepper and red pepper, respectively. The reason for this result can be explained by surface texture. Vega-Gálvez et al. (2008) and Schnabel et al. (2012) reported that the SEM images of dried red and whole black pepper, respectively. Red pepper has a rougher surface than that of black pepper which can provide protective sites for pathogens to avoid or evade vacuumed hydrogen peroxide vapor treatment. Also, ground red pepper consists of overlapping layers which make contact with each other and could hinder the penetration effect of vacuumed hydrogen peroxide vapor. There are several studies which support this hypothesis. Kim et al. (2014) reported that *B. cereus* spores on smooth polystyrene were inactivated by 3.9 log CFU/cm^2 following cold plasma treatment at 900 W with a He-O_2 mixture whereas spores in red pepper powder were not reduced using the same treatment. Schnabel et al. (2012) also reported that decontamination of *Bacillus atrophaeus* on a variety of seeds by microwave driven discharge processed gas differed depending on seed surface. *B. atrophaeus* in whole black pepper, which had the most complex surface among seeds tested showed the most resistance to plasma treatment.

The effect of hydrogen peroxide on food quality is usually negative because it is primarily a bleaching agent. There have been several studies which confirm color or quality changes during hydrogen peroxide treatment. Alexandre et al. (2012) reported that 5% hydrogen peroxide treatment for 2 min on strawberries, watercress and red bell peppers resulted in color changes. Also 5% hydrogen peroxide reduced total anthocyanins. McWatters et al. (2002) reported that 2% hydrogen peroxide treatment at 50°C for 60 s changed color values and sensory quality of lettuce. But HPV treatment on lettuce did not change color values and maximum load (Back et al., 2014). Also, Back et al. (2014) reported that residual hydrogen peroxide in leachate from lettuce treated with 10% HPV for 10 min was reduced to under the detection limit after 36 h storage at 4°C. In the present study vacuumed hydrogen peroxide vapor also did not affect color values. Moreover, I did not find residual hydrogen peroxide in foods though the total volume of hydrogen peroxide (50%, 0.5 ml) used in this study was admittedly smaller than that of Back et al. (2014).

In conclusion, I can recommend vacuumed hydrogen peroxide vapor as a pasteurization intervention for black and red pepper. Vacuumed hydrogen peroxide vapor for 1 min reduced *E. coli* O157:H7 and *S.* Typhimurium in black and red pepper by > 5.34 and 4.52 log CFU/g and 3.01 and 2.36 log CFU/g, respectively. Also 50% VHPV treatment reduced total aerobic bacteria in black and red pepper by 2~3 log CFU/g (data not shown). Vacuumed hydrogen peroxide vapor is more effective on black pepper rather than red pepper because of surface differences. So, applying vacuumed hydrogen peroxide vapor before red pepper is ground or fully crushed is recommended. Further investigation is needed to confirm levels of residual hydrogen peroxide after vacuumed hydrogen peroxide vapor treatment.

Chapter IV.

Inactivation of foodborne pathogens in low a_w foods by combining thermal and non-thermal treatment

A part from this chapter were published in

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IV-1. Inactivation of foodborne pathogens in apple juice concentrates with different soluble solids content by combining ozone treatment with mild heat

IV-1.1. Introduction

Humankind has enjoyed apples since ancient times. Not only because of their taste, sweetness, and texture, but also for their health benefits, as shown in phrase “an apple a day keeps the doctor away”. Because apples have antioxidant activity and a cholesterol lowering effect, apple consumption can reduce the risk of cancer, heart disease, asthma, and type II diabetes. Also, they have the positive effects of increasing lung function and weight loss (Boyer and Liu, 2004). These healthful benefits also operate when apple juice is consumed. Apple juice contains antioxidants, iron, vitamin C, and low levels of sodium, cholesterol and fat (Patil et al., 2010a). It is also reported that apple juice has beneficial effects on degenerative diseases, and protective effects against cardiovascular diseases and cancer (Torres et al., 2011). Also, apple juice consumption has increased continuously for more than 30 years (USDA, 2012).

However, several outbreaks have been caused by pathogens in fruit juices. In 1996, there was a large outbreak of *E. coli* O157:H7 traced to

unpasteurized commercial apple juice, resulting in 56 illnesses, 25 hospitalizations, 14 people developing the hemolytic uremic syndrome and one death (Cody et al., 1999). From 1995 to 2005 there were 21 outbreaks associated with juice products; ten of these were caused by apple juice (Vojdani et al., 2008). Furthermore, *Salmonella* showing increased heat and acid resistance was isolated from unpasteurized juice (Sharma et al., 2005).

For storage and transportation, juice is usually condensed to a high solids content. Juice concentrates have low pH which is an extreme challenge for pathogen survival. However, pathogens can contaminate juices during extraction and post processing (Enache et al., 2006). There was one study which confirmed the survival of pathogens in juice of high solids content (Oyarzábal et al., 2003). Because of juice concentrate's low pH, pathogens can adapt to an acidic environment. Acid-adapted pathogens also exhibit enhanced heat resistance and increased D-value. Thus, it is extremely hard to kill pathogens in juice concentrate by thermal treatment (Mazzotta, 2001; Enache et al., 2006). Therefore, an improved method is needed to kill pathogens in high solids content juices.

Ozone is a triatomic allotrope of oxygen with blue color and has great oxidation potential (Patil et al., 2009). Because of this oxidation potential, ozone attacks numerous components of microorganisms. Scott and Lesher (1963) reported that ozone attacked the double bonds of lipids in the cell membrane, so cell leakage or cell lysis occurred. Also, ozone inactivates enzymes by the oxidation of sulfhydryl groups, produces DNA lesions and inhibits transcriptional ability (Chang, 1971; Mura and Chung, 1990). In 2001, the gaseous and aqueous phases of ozone were approved by the FDA as a direct food additive for the treatment, storage and processing of foods (Khadre et al., 2001). This approval led many food researchers to apply ozone to fruit juices to inactivate pathogens (Williams et al., 2004; Patil et al., 2009; Choi et al. 2012).

The recent concept of hurdle technology for inactivating pathogens in apple juice needs to be investigated. Williams et al. (2004) reported that ozone treatment of apple juice with mild heat (50°C) reduced foodborne pathogens more rapidly than ozone treatment alone. However, there have been very few research studies investigating the bactericidal effect when

apple juice is treated with both heat and ozone gas simultaneously and their effect on quality changes of juice concentrate. Therefore this study evaluates the bactericidal effect of the combination of ozone and heat treatment on three concentrations of apple juice (12, 18, 36 and 72 °Brix). Twelve, 18 and 36 °Brix apple juice were used to investigate the condition of synergistic effect of ozone and heat treatment on apple juice. To confirm quality changes occurring during the combination treatment, color values and residual ozone concentration were measured.

IV-1.2. Materials and Methods

Preparation of bacterial strains and inoculums Strains of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890) and *S.* Typhimurium (ATCC 19585, ATCC 43971, ATCC 700408) were obtained from the School of Food Science bacterial culture collection of Seoul National University (Seoul, Korea). Stock cultures were maintained by combining 0.7 ml of 24 h cultures in Tryptic Soy Broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) and 0.3 ml of 50% glycerol and storing at –80°C. Working cultures were streaked onto Tryptic Soy Agar (TSA; Difco), incubated at 37°C for 24 h, and stored at 4°C before use. Each strain of *E. coli* O157:H7 and *S.* Typhimurium was incubated in 5 ml of TSB at 37°C for 24 h, harvested by centrifugation at 4000 x g at 4°C for 20 min and washed twice with sterile 0.2% peptone (Bacto, Sparks, MD) water. The final pellets, corresponding to approximately 10^8-10^9 CFU/ml, were obtained through resuspension in sterile 0.2% peptone water. The cell concentration was determined by plating aseptically on TSA and incubating

at 37°C for 24 h. Mixed culture cocktails were prepared by blending equal volumes of each test strain.

Sample preparation and inoculation Apple juice (72 °Brix, a_w 0.75) was purchased at a local super market (Chung-Book, Korea) and stored at 4°C before use. Thirty-six (a_w 0.94), 18 (a_w 0.98) and 12 °Brix (a_w 0.99) apple juices were prepared by diluting 72 °Brix apple juice with sterile distilled water. Soluble solids content was measured by a digital refractometer (Atago Co., Ltd.). Two hundred ml of apple juice was dispensed into a 500 ml bottle and 0.2 ml of antifoam B Emulsion (Sigma Aldrich, Ireland Ltd.) was added to apple juice to prevent excessive foaming. Then, 0.1 ml of mixed culture cocktail (*E. coli* O157:H7 and *S.* Typhimurium) was inoculated into apple juice when the preset temperature (25, 45 and 50°C) in the water bath stabilized. Apple juice was shaken vigorously by hand for 10 seconds for equilibration of inoculum in apple juice. The final cell concentration in apple juice was approximately 10^5-10^6 CFU/ml.

Ozone and heat treatment The apparatus used in this study was shown in Fig. III -1 (Sung et al., 2014). An ozone generator (Ozonetech Co., Ltd, Korea) was used to produce gaseous ozone from ambient air at generation rates of 2.0-3.0 g/m^3 and a flow rate of 3.0 L/min. The concentration of ozone was continuously monitored with an ozone monitor (Okitrotec Co., Japan). Ozone was directly injected into 500 ml bottles containing juice samples through a delivery tube and a perforated tube for sparging. When target temperatures (25, 45 and 50°C) were attained in a shaking water bath, 0.1 ml of mixed culture cocktail was inoculated into apple juice and heat treatments were performed. Juice samples treated with heat alone were designated as the heat treatment group. Apple juice samples treated at 25°C without or with ozone were designated as controls for confirming the effect of heat or ozone at 25°C, respectively. Treatment times were 20, 40 or 60 s for all samples. Juice samples were treated by mixing at 150 rpm in a shaking water bath during the entire treatment time for even distribution of inoculum and dispersal of ozone. All experiments were performed in a fume hood. An ozone decomposer was used for decomposition of excess ozone.

Bacteriological analysis After treatment, sample aliquots (1 ml) were transferred into test tubes containing 9 ml of D/E neutralizing broth (Difco, Becton Dickinson, Sparks, MD, USA) and homogenized using a vortex mixer (VM-10, Daihan Scientific co., Ltd, Korea) for bacteriological analysis of pathogens. Samples were 10-fold serially diluted with 9 ml of sterile 0.2% peptone and 0.1 ml of samples was spread plated onto selective media. Sorbitol MacConkey Agar (SMAC; Difco) and Xylose Lysine Desoxycholate Agar (XLD; Difco) were used as selective media for enumeration of *E. coli* O157:H7 and *S.* Typhimurium, respectively. Where low populations of surviving cells were anticipated, 1 ml aliquots of the original homogenate in D/E neutralizing broth were equally distributed onto four plates of each selective medium and spread-plated. All plates were incubated at 37°C for 24 h and colonies enumerated. The detection limit was 1.0 log CFU/ml.

Color measurement Color of apple juice was measured by using a Minolta colorimeter (model CR400; Minolta Co., Osaka, Japan). The

L, *a* and *b* values which indicate lightness, redness and yellowness, respectively, were measured to investigate the color changes of apple juice after each heat and/or ozone treatment. Untreated apple juice was used as the control. Before measurement, treated juice was cooled to about 15°C by dipping the bottle in crushed ice. Two ml of sample was poured into the bottom half of the measurement equipment. The measuring head of the colorimeter was placed on top of the measurement equipment. All measurements were conducted in triplicate.

Residual ozone measurement To measure ozone concentration, distilled water was substituted for apple juice, as will be explained in the discussion. The ozone concentration of distilled water treated with ozone in the same way as apple juice was measured by the Indigo method (Bader et al., 1981). An indigo stock reagent was prepared by the following method (Gordon et al., 2002). Indigo stock solution was prepared by dissolving 770 mg of potassium indigotrisulfonate (Sigma Aldrich Co., LLC) into a 1 l flask containing 500 ml of distilled water and 1 ml of phosphoric acid (85%)

and diluting to volume (1 l) with distilled water. Indigo reagent II was prepared in a 1 l flask by adding 100 ml of indigo stock solution, 10 g of sodium dihydrogen phosphate (NaH_2PO_4) and 7 ml of phosphoric acid (85%). This was stirred and diluted to volume (1 l) with distilled water. The solution was stored in the dark. After each treatment, the treated sample was cooled to about 15°C and then 90 ml of sample was transferred to a flask containing 10 ml of indigo reagent II. The absorbance was measured by a spectrophotometer at 600 nm. Residual ozone in treated samples was obtained by the following equation,

$$\mathrm{mg}\frac{O_3}{L} = \frac{100 \times A}{f \times b \times V}$$

where A is the difference of absorbance between the blank solution (control) and treated sample, f is an indigo sensitivity coefficient (0.42), b is the path length of the cell (1 cm), and V is the volume of the sample (ml). The blank solution was untreated distilled water.

Statistical analysis All experiments were duplicate-plated and replicated three times. All data were analyzed with one-way ANOVA using Statistical Analysis System (SAS Institute, Cary, NC, USA) and Duncan`s multiple range test to investigate if there were significant differences ($P < 0.05$) in mean values of microorganism populations and colors. Microbial counts were transformed to $\log_{10}$ values for analysis. One log was used for calculations where populations were reduced to under the detection limit.

IV-1.3. Results

Initial populations of *E. coli* O157:H7 and *S.* Typhimurium in inoculated apple juice were approximately 10^5-10^6 CFU/ml and the detection limit was 1.0 log CFU/ml. The combination of ozone and heat treatment exhibited a great effect in reducing *E. coli* O157:H7 and *S.* Typhimurium in apple juice. Fig. IV-2 through IV-9 represent the populations of surviving *E. coli* O157:H7 and *S.* Typhimurium in each of four different concentrations (12, 18, 36, 72 °Brix) of apple juice after treatments, respectively.

Fig. IV-2, IV-3, IV-4 and IV-5 show the bactericidal effect of the combination treatment of ozone and heat against *E. coli* O157:H7 in apple juice of 12, 18, 36, 72 °Brix. The levels of surviving pathogens were reduced in all treated apple juice samples as treatment temperature increased from 25 to 50°C. Counts of *E. coli* O157:H7 in 12 °Brix apple juice treated only with heat (25, 45 and 50°C) for 1 min were reduced by 0.20, 0.37 and 2.16 log CFU/ml, respectively. In the case of the combination treatment of ozone and heat for 1 min, *E. coli* O157:H7 in 12 °Brix apple juice was

reduced by 1.50 and 1.60 log CFU/ml at 25 and 45°C, respectively, and was below detection limit after treatment at 50°C (Fig. IV-2).

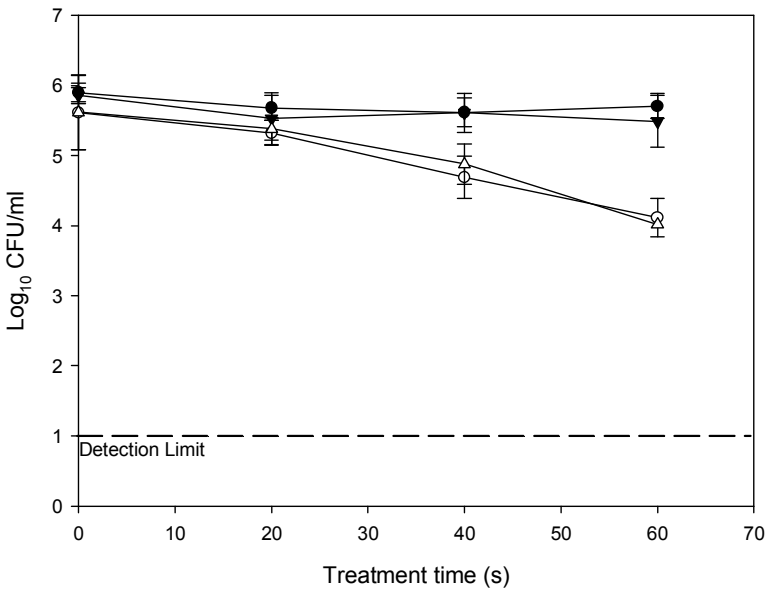
When 18 °Brix apple juice was treated with heat alone (45 and 50°C) for 1 min, populations of *E. coli* O157:H7 were decreased by 1.29 and 2.36 log CFU/ml, respectively. The combined treatment of heat with ozone for 1 min inactivated *E. coli* O157:H7 by 1.74 and 3.86 log CFU/ml at 25 and 45°C, respectively. In the case of the combination treatment at 50°C, surviving populations were reduced to below the detection limit (1.0 log CFU/ml) (Fig. IV-3).

In 36 °Brix apple juice, pathogen populations were reduced by 1.56 and 2.58 log CFU/ml after heat treatment for 1 min at 45 and 50°C, respectively. Populations were reduced by 0.93 and 2.49 log CFU/ml, respectively, when ozone and heat (25 and 45°C) were simultaneously applied to apple juice for 1 min. Pathogens were reduced to below the detection limit after combination treatments at 50°C for 1 min (Fig. IV-4).

The surviving populations of *E. coli* O157:H7 in 72 °Brix apple juice were reduced by 1.66 and 4.18 log CFU/ml at 45 and 50°C, respectively.

Regarding the combination treatment for 1 min, populations were decreased by 1.13 and 2.49 log CFU/ml at 25 and 45°C, respectively, and to below the detection limit at 50°C (Fig. IV-5).

(a)



(b)

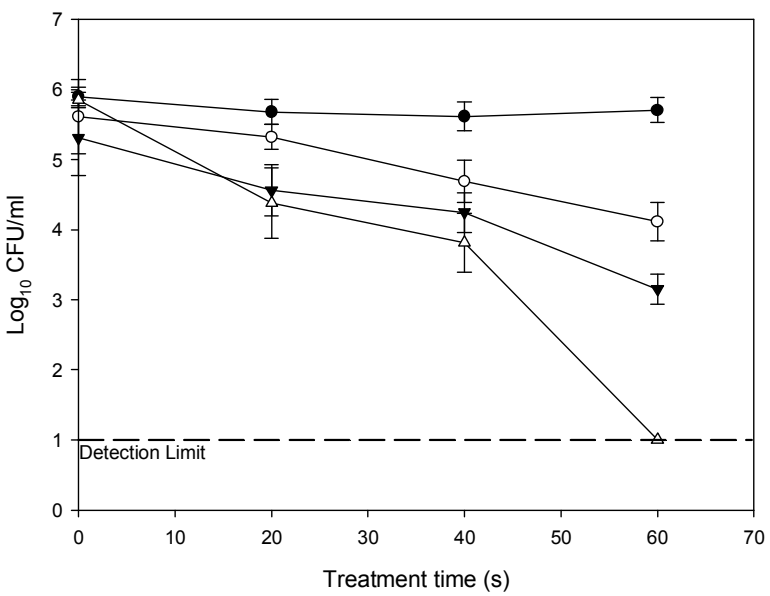
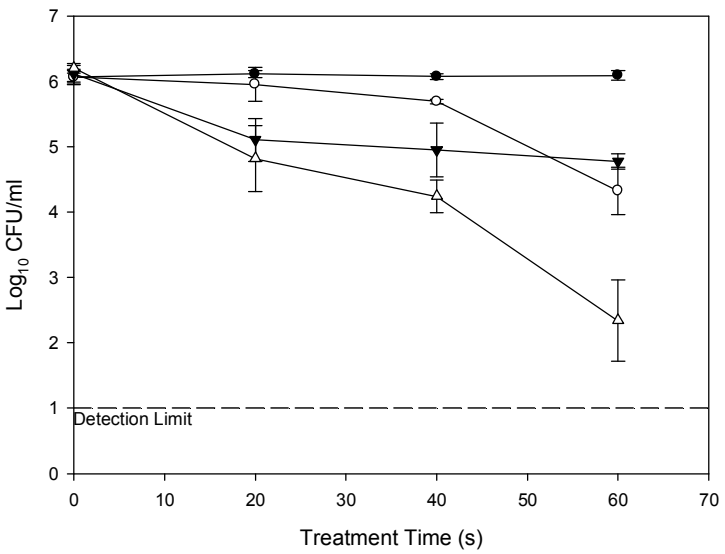


Figure IV-1. Inactivation of *E. coli* O157:H7 in 12 °Brix apple juice treated with heat and ozone. (a) ● 25°C; ○ 25°C + ozone; ▼ 45°C; ∆ 45°C + ozone, (b) ● 25°C; ○ 25°C + ozone; ▼ 50°C; ∆ 50°C + ozone.

(a)



(b)

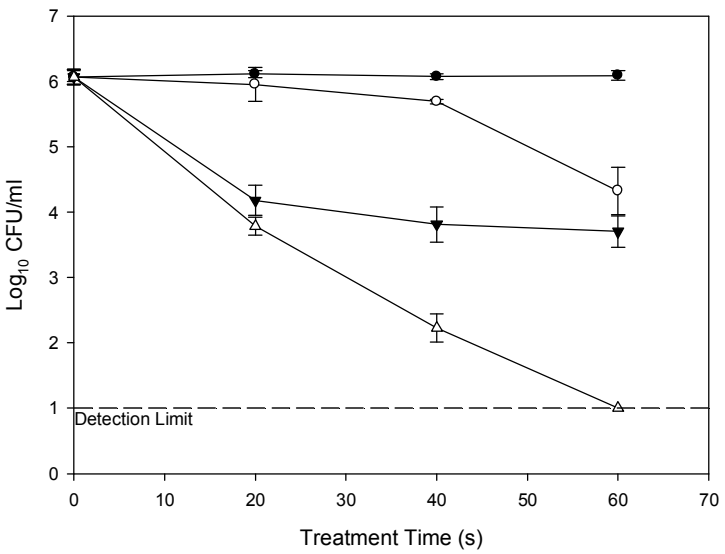
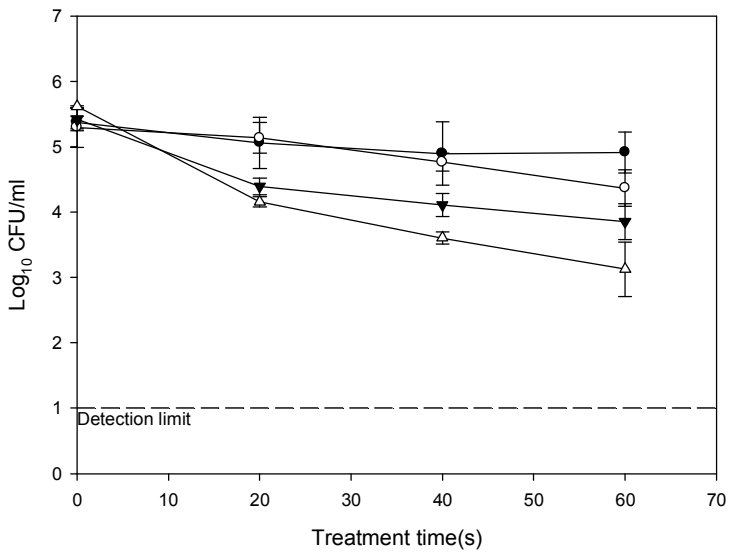


Figure IV-2. Inactivation of *E. coli* O157:H7 in 18 °Brix apple juice treated with heat and ozone. (a) ● 25°C; ○ 25°C + ozone; ▼ 45°C; ∆ 45°C + ozone, (b) ● 25°C; ○ 25°C + ozone; ▼ 50°C; ∆ 50°C + ozone.

(a)



(b)

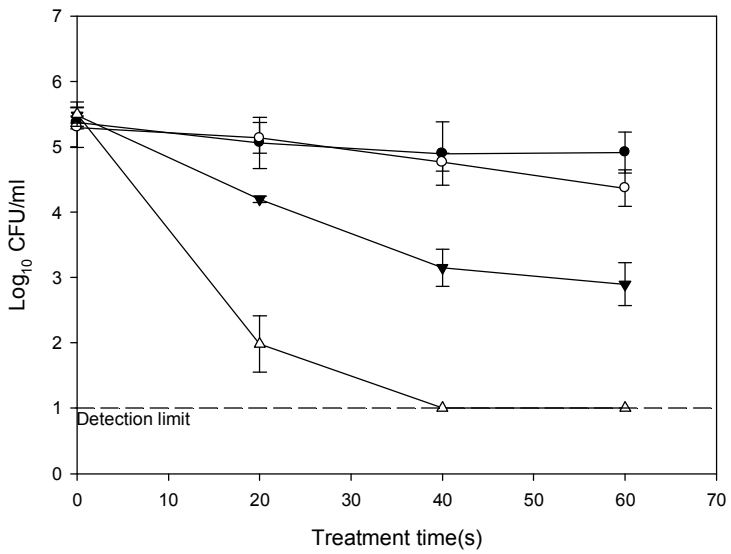
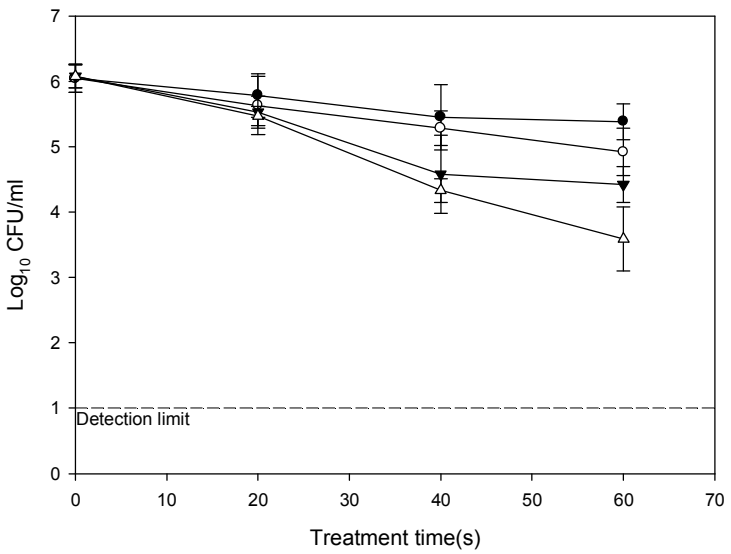


Figure IV-3. Inactivation of *E. coli* O157:H7 in 36 °Brix apple juice treated with heat and ozone. (a) ● 25°C; ○ 25°C + ozone; ▼ 45°C; ▵ 45°C + ozone, (b) ● 25°C; ○ 25°C + ozone; ▼ 50°C; ▵ 50°C + ozone.

(a)



(b)

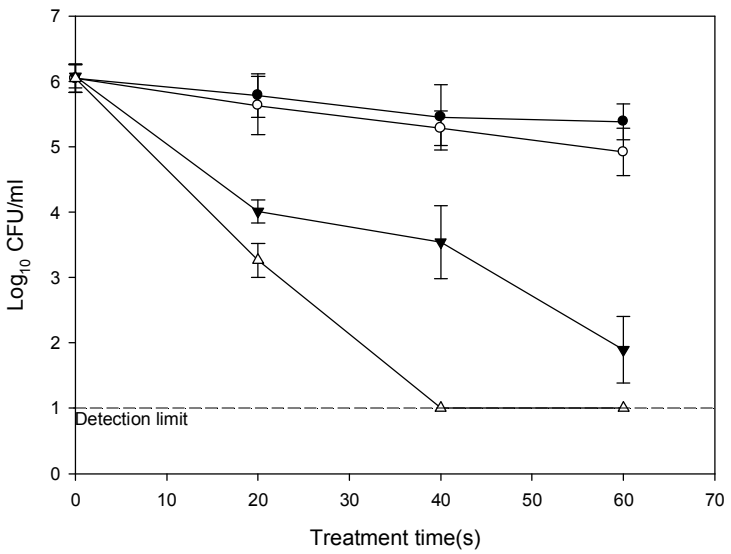


Figure IV-4. Inactivation of *E. coli* O157:H7 in 72 °Brix apple juice treated with heat and ozone. (a) ● 25°C; ○ 25°C + ozone; ▼ 45°C; ∆ 45°C + ozone, (b) ● 25°C; ○ 25°C + ozone; ▼ 50°C; ∆ 50°C + ozone.

The reduction of *S.* Typhimurium in 12, 18, 36, and 72 °Brix apple juice after the combination treatment of ozone and heat is shown in Fig. IV-6, IV-7, IV-8 and IV-9. The reduction trend was similar to that of *E. coli* O157:H7. The reduction of *S.* Typhimurium in 12 °Brix apple juice is shown in Fig. III-5. The population of surviving pathogens (25, 45 and 50°C) was decreased to 0.09, 0.62 and 2.50 log CFU/ml after 1 min heat treatment alone. In apple juice treated with both ozone and heat, *S.* Typhimurium was reduced by 1.84, 2.20 log CFU/ml at 25, 45°C and below the detection limit at 50°C (Fig. IV-6).

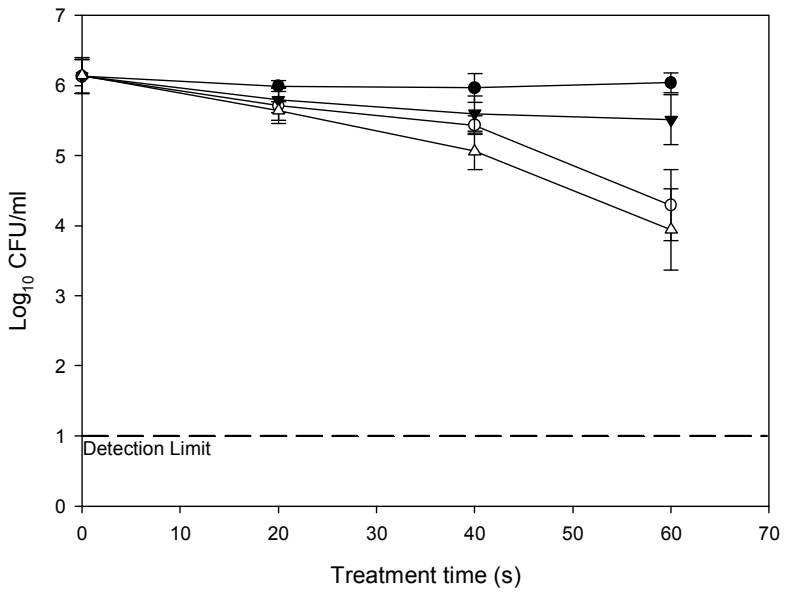
Levels of surviving populations were decreased by 1.45 and 4.02 log CFU/ml in 18 °Brix apple juice after 1 min heat treatment at 45, 50°C, respectively. In apple juice treated with ozone for 1 min, *S.* Typhimurium was decreased by 1.38 and 2.70 log CFU/ml at 25 and 45°C, respectively. After ozone treatment at 50°C, populations were reduced to below the detection limit (Fig. IV-7).

In 36 °Brix apple juice, heat treatment of 45 and 50°C for 1 min inactivated 1.60 and 3.03 log CFU/ml of this pathogen, respectively. The

combined treatment of heat with ozone for 1 min inactivated 0.74 and 2.83 log CFU/ml of *S.* Typhimurium at 25 and 45°C, respectively. Populations were reduced to below the detection limit at 50°C (Fig. IV-8).

Surviving populations of *S.* Typhimurium in 72 °Brix apple juice were reduced by 2.09 and 4.03 log CFU/ml after heat treatment at 45 and 50°C for 1 min, respectively. In the case of the combination treatment for 1 min, populations were decreased by 1.14 and 2.80 log CFU/ml at 25 and 45°C and to below the detection limit at 50°C (Fig. IV-9).

(a)



(b)

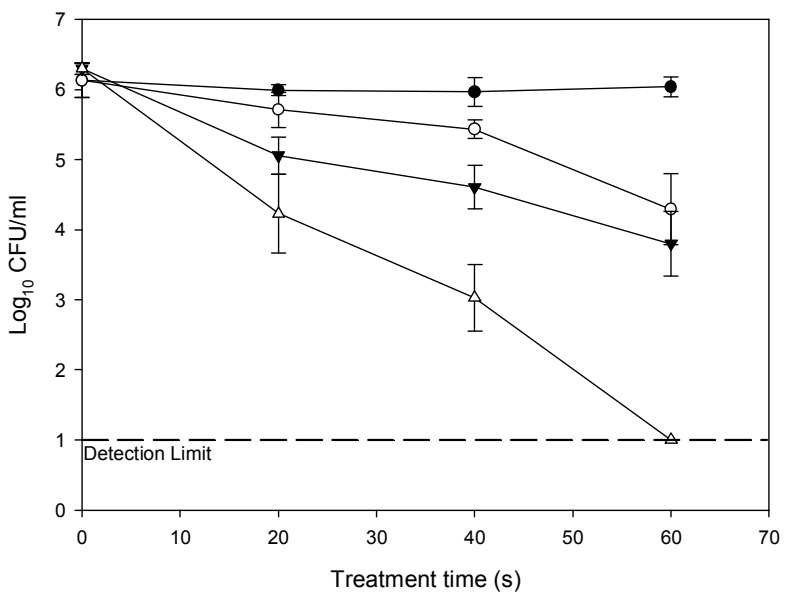
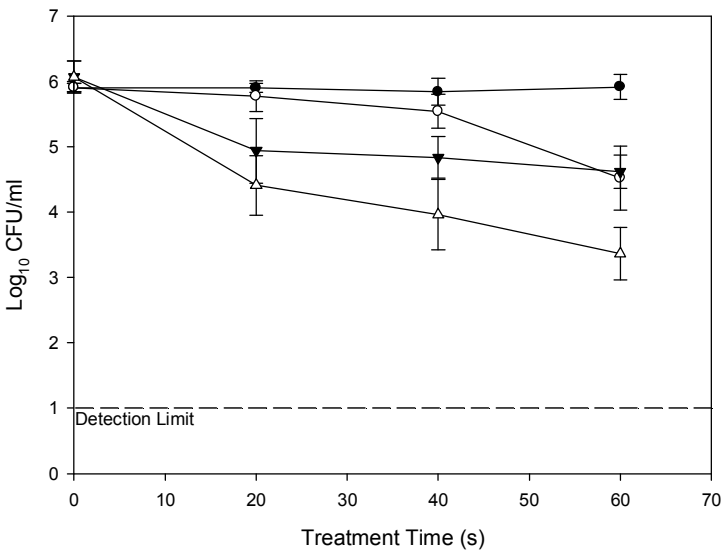


Figure IV-5. Inactivation of *S.* Typhimurium in 12 °Brix apple juice treated with heat and ozone. (a) ● 25°C; ○ 25°C + ozone; ▼ 45°C; ∆ 45°C + ozone, (b) ● 25°C; ○ 25°C + ozone; ▼ 50°C; ∆ 50°C + ozone.

(a)



(b)

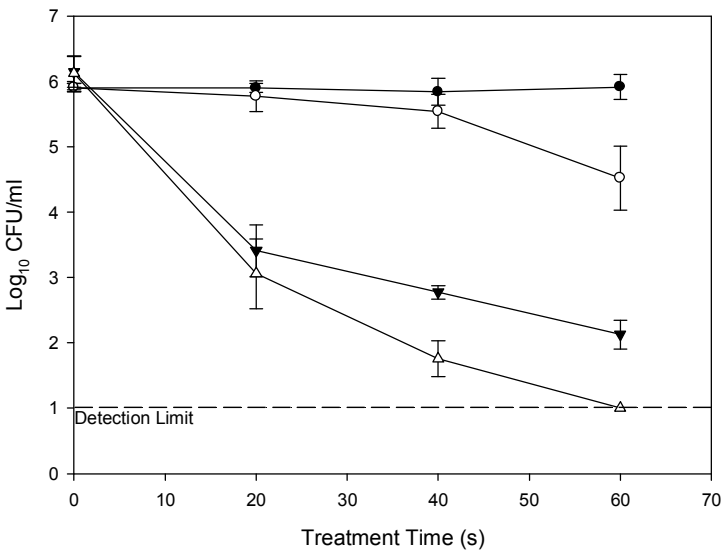
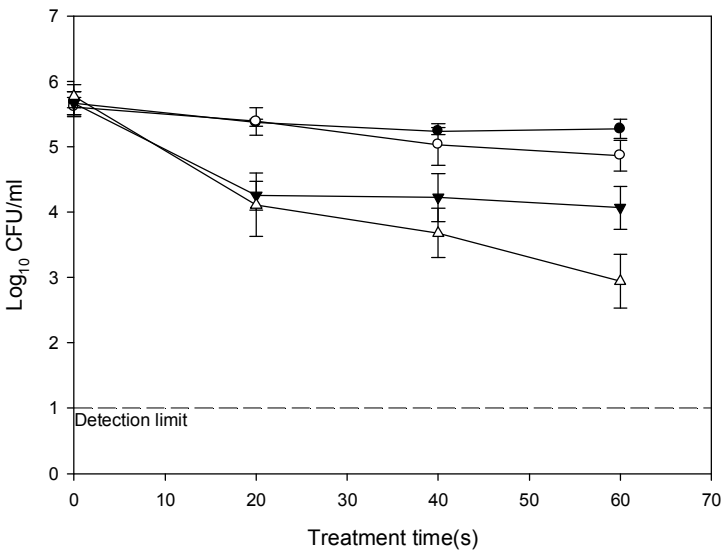


Figure IV-6. Inactivation of *S.* Typhimurium in 18 °Brix apple juice treated with heat and ozone. (a) ● 25°C; ○ 25°C + ozone; ▼ 45°C; ∆ 45°C + ozone, (b) ● 25°C; ○ 25°C + ozone; ▼ 50°C; ∆ 50°C + ozone.

(a)



(b)

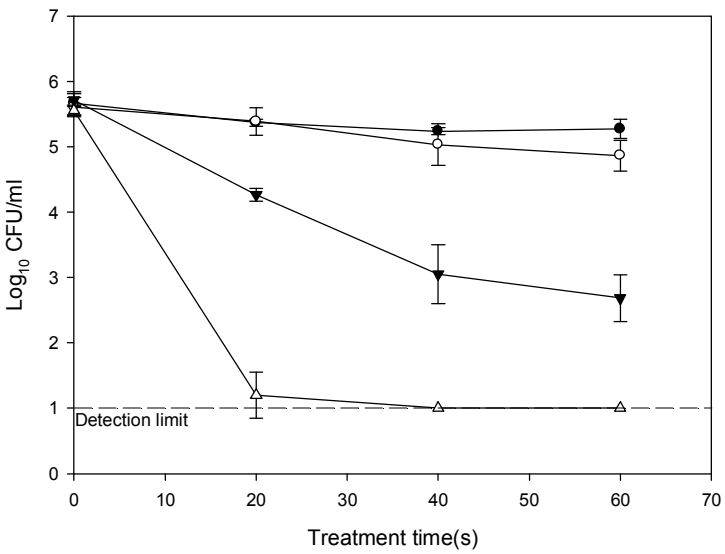
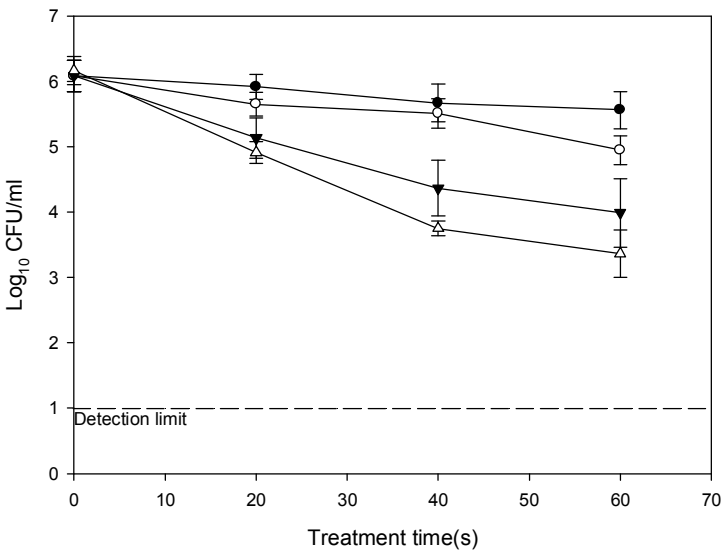


Figure IV-7. Inactivation of *S.* Typhimurium in 36 °Brix apple juice treated with heat and ozone. (a) ● 25°C; ○ 25°C + ozone; ▼ 45°C; ∆ 45°C + ozone, (b) ● 25°C; ○ 25°C + ozone; ▼ 50°C; ∆ 50°C + ozone.

(a)



(b)

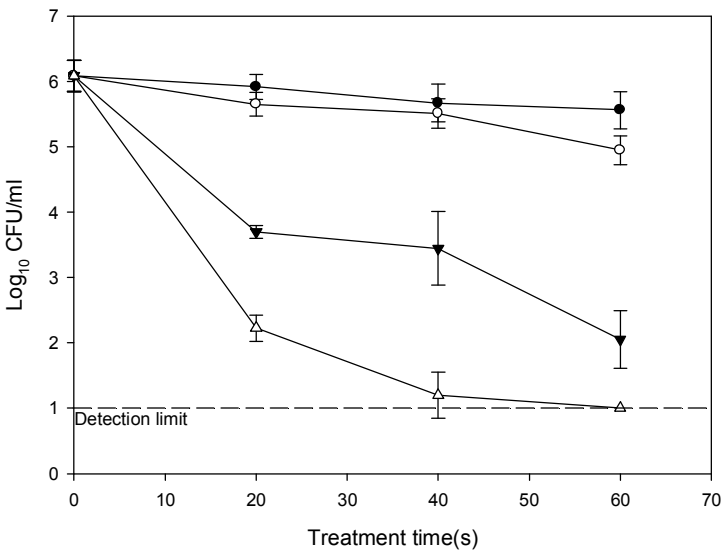


Figure IV-8. Inactivation of *S.* Typhimurium in 72 °Brix apple juice treated with heat and ozone. (a) ● 25°C; ○ 25°C + ozone; ▼ 45°C; ▵ 45°C + ozone, (b) ● 25°C; ○ 25°C + ozone; ▼ 50°C; ▵ 50°C + ozone.

The Hunter color values of apple juice of 12, 18, 36, and 72 °Brix treated with heat alone or with the combination of ozone and heat are shown in Tables IV-1 and IV -2. In all treated apple juice samples of 12, 18, 36 and 72 °Brix, *L*, *a*, and *b* values were not significantly different ($P > 0.05$) from those of the control. The combination treatment of both heat and ozone did not significantly affect the color value of apple juice.

Residual ozone was measured by the Indigo method after ozone treatment on distilled water for 1 min at each temperature (25, 45 and 50°C). As shown in Table IV-3, in all ozone treated samples, the concentrations of residual ozone were in the range from 0.11 to 0.31 mg/L when treated at 25, 45 and 50°C, respectively. Also, the concentration of ozone decreased as the treatment temperature increased.

Table IV-1. Hunter's color *L* (lightness), *a* (redness) and *b* (Yellowness) values[a] of 12 and 18 °Brix apple juice treated simultaneously with gaseous ozone and mild heat.

Soluble solid content (°Brix)	Treatment	Parameter		
		L	*a*	*b*
12	Control	26.40 ± 2.91	0.28 ± 0.03	3.94 ± 0.24
	25 °C	26.61 ± 0.59	0.25 ± 0.03	3.95 ± 0.11
	25 °C, ozone	26.93 ± 0.91	0.24 ± 0.02	3.78 ± 0.12
	45 °C	27.27 ± 1.77	0.25 ± 0.03	3.92 ± 0.06
	45 °C, ozone	27.07 ± 1.26	0.24 ± 0.03	3.90 ± 0.04
	50 °C	27.03 ± 1.68	0.22 ± 0.05	4.00 ± 0.06
	50 °C, ozone	27.54 ± 1.02	0.23 ± 0.06	3.83 ± 0.13
18	Control	25.02 ± 0.24	0.27 ± 0.06	3.89 ± 0.61
	25 °C	25.35 ± 0.35	0.30 ± 0.04	4.11 ± 0.60
	25 °C, ozone	25.39 ± 0.07	0.28 ± 0.06	4.10 ± 0.33
	45 °C	25.08 ± 0.01	0.27 ± 0.02	3.96 ± 0.43
	45 °C, ozone	25.24 ± 0.08	0.30 ± 0.07	3.40 ± 0.24
	50 °C	25.20 ± 0.32	0.38 ± 0.03	3.23 ± 0.40
	50 °C, ozone	25.44 ± 0.08	0.31 ± 0.04	3.46 ± 0.76

[a] Mean of three replications ± standard deviation. Mean values in same column did not differ significantly ($P > 0.05$).

Table IV-2. Hunter's color *L* (lightness), *a* (redness) and *b* (Yellowness) values[a] of 36 and 72 °Brix apple juice treated simultaneously with gaseous ozone and mild heat.

Soluble solid content (°Brix)	Treatment	Parameter		
		L	*a*	*b*
12	Control	25.81 ± 0.32	0.29 ± 0.09	4.36 ± 0.29
	25 °C	25.57 ± 0.24	0.28 ± 0.07	4.61 ± 0.15
	25 °C, ozone	25.53 ± 0.22	0.31 ± 0.13	4.55 ± 0.31
	45 °C	25.41 ± 0.21	0.24 ± 0.11	4.67 ± 0.22
	45 °C, ozone	25.65 ± 0.19	0.29 ± 0.12	4.43 ± 0.57
	50 °C	25.63 ± 0.46	0.28 ± 0.06	4.76 ± 0.09
	50 °C, ozone	25.26 ± 0.15	0.25 ± 0.06	4.77 ± 0.16
18	Control	27.52 ± 0.14	0.30 ± 0.04	4.06 ± 0.06
	25 °C	27.65 ± 0.41	0.27 ± 0.03	4.01 ± 0.08
	25 °C, ozone	27.93 ± 0.88	0.25 ± 0.05	4.20 ± 0.16
	45 °C	28.11 ± 0.90	0.30 ± 0.10	4.15 ± 0.19
	45 °C, ozone	28.24 ± 1.26	0.34 ± 0.16	3.91 ± 0.11
	50 °C	27.90 ± 0.72	0.34 ± 0.06	3.60 ± 0.78
	50 °C, ozone	27.69 ± 1.24	0.28 ± 0.16	3.90 ± 0.47

[a] Mean of three replications ± standard deviation. Mean values in same column did not differ significantly ($P > 0.05$).

Table III-3. Residual ozone in distilled water stored at 4°C for 6 h after ozone and heat treatment.

Treatment	Residual Ozone[a] (mg O_3/L)
25°C, ozone	0.306±0.269 A
45°C, ozone	0.141±0.076 B
50°C, ozone	0.111±0.062 BC

[a] Mean values ± standard deviation. Mean values in the same column followed by different superscript are significantly different ($P< 0.05$).

IV-1.4. Discussion

Apple juice is of low pH (3.1-4.4) and thus formerly was considered to be hostile to the survival of foodborne pathogens (Mattick and Moyer, 1983). But several studies have confirmed numerous outbreaks associated with the consumption of apple juice (Goverd et al., 1979; Steele et al., 1982; Parish, 1997; Sivapalasingam et al., 2004; Rangel et al., 2005; Vojdani et al., 2008). Conventional heating is the usual intervention performed for pasteurization of apple juice. But thermal treatment leads to quality deterioration of apple juice. Natural flavoring compounds found in apple juice are destroyed by conventional heat treatment. During high-temperature short-time (HTST) treatment more than 50% of acetic acid, hexanal, ethyl acetate, ethyl butyrate, methyl butyrate and 1-hexanal are lost. Also, 36% of butyl hexanoate and 22% of hexyl acetate are lost to evaporation (Aguilar-Rosas et al., 2007). Moreover during HTST treatment, turbidity and lightness of cloudy apple juice are increased (Krapfenbauer et al., 2006).

Because conventional heating has many disadvantages, numerous other

non-thermal treatments have been evaluated for inactivation of pathogens in apple juices. Yuste and Fung (2004) used nisin and cinnamon to rapidly reduce *S.* Typhimurium and *E. coli* O157:H7 in apple juice. Buchanan et al. (1998) used gamma irradiation for inactivating *E. coli* O157:H7 in apple juice and reported that the D value of apple juice is in the range of 0.26-0.35 kGy. Also, dense phase carbon dioxide (Liao et al., 2007), high hydrostatic pressure (Teo et al., 2001), ultraviolet (Gachovska et al., 2008; Keyser et al. 2008) and pulsed electric fields (Evrendilek et al., 1999; Gachovska et al., 2008) were also tested to control pathogens in apple juice.

Ozone is one of the most popular non-thermal treatments to reduce pathogens in foods. There are studies which confirm the antimicrobial effect and influence of ozone on quality in juice. Patil et al. (2009) reported the bactericidal effect of ozone treatment on model orange juice. There was a 6 log CFU/ml reduction of *E. coli* during 60 s ozone treatment with 0.12L/min flow rate and 0.075-0.078 mg/ml of ozone concentration. Steenstrup and Floros (2004) reported the effect of ozone (860 ppm) and low temperature on inactivation of *E. coli* O157:H7 in apple cider. D-values of *E. coli*

O157:H7 ranged from 0.6 and 1.5 min at 20°C and 5°C. Anthocyanin and ascorbic acid degradation of strawberry juice by ozone treatment was also reported (Tiwari et al., 2009b).

In this study it was tested the effect of ozone treatment with a mild heat for inactivation of pathogens in apple juice. The combination of ozone and heat treatment showed great efficacy for reducing pathogens in apple juice with different soluble solids content. Williams et al. (2004) reported inactivation of *E. coli* O157:H7 and *Salmonella* in orange juice by applying ozone at various treatment temperatures. Mild heat treatment combined with ozone showed a much better bactericidal effect than ozone treatment alone. To reduce pathogen levels to below the detection limit, more than 240 min was required for *E. coli* O157:H7 and *Salmonella* at 4°C but at 50°C, 45 min for *E. coli* O157:H7 and 15 min for *Salmonella* was necessary. This tendency was also observed in my study. When juices were treated with ozone alone, 0.74 to 1.73 log CFU/ml reductions occurred after 1 min. But ozone treatment combined with heat at 50 or 55°C reduced pathogens to under the detection limit after less than 1 min.

Choi et al. (2012) reported on the efficacy of ozone to inactivate pathogens in apple juice of different levels of soluble solids (°Brix). When 18 and 36 °Brix apple juice was treated with ozone, *E. coli* O157:H7 was reduced to under the detection limit after 10 min. *S.* Typhimurium and *L. monocytogenes* also showed a similar tendency. But in the case of 72 °Brix apple juice concentrate, ozone produced 0.41-1.73 log reductions of all 3 pathogens during 10 min of treatment. This indicates that ozone treatment alone is not suitable for high solids content juice pasteurization. However, in this study, using the combination of ozone and heat treatment, I was able to reduce pathogens to under the detection limit after 1 min treatment in 72 °Brix apple juice. The combination of ozone and heat treatment can be used as a pasteurization intervention for high solids content juices.

The results of this study demonstrated that ozone combined with heat shows great antimicrobial effect when applied to apple juice of different solids content. Especially in some cases I obtained a greater effect than expected. Tables IV-4 and IV-5 show the comparison of the sum of reductions and the combination effect on *E. coli* O157:H7 and *S.*

Typhimurium. The combined treatment showed a synergistic effect on *E. coli* O157:H7 at 45 and 50°C in 18 °Brix apple juice and at 50°C in 12 and 36 °Brix apple juice, respectively. In the case of *S.* Typhimurium, the combined treatment showed a synergistic effect at 50°C in 12 and 36 °Brix apple juice. The combined treatment did not show any synergistic effect on *E. coli* O157:H7 and *S.* Typhimurium in 72 °Brix apple juice.

Table IV-4. Comparison of reductions of *E. coli* O157:H7 in apple juice by heat or ozone alone and the combination treatment.

°Brix	Treatment temperature (°C)	Heat	Ozone (at 25°C)	Sum	Combination
12	45	0.37 ± 0.30[a]	1.50 ± 0.45	1.87 ± 0.39A	0.96 ± 0.52A
	50	1.68 ± 0.33	1.50 ± 0.45	3.18 ± 0.14B	4.85 ± 0.11A
18	45	1.34 ± 0.12	1.65 ± 0.38	2.99 ± 0.38B	3.87 ± 0.52A
	50	2.36 ± 0.14	1.65 ± 0.38	4.02 ± 0.30B	5.07 ± 0.12A
36	45	1.56 ± 0.69	0.93 ± 0.44	2.49 ± 0.35A	2.49 ± 0.43A
	50	2.58 ± 0.39	0.93 ± 0.44	3.51 ± 0.20B	4.49 ± 0.19A
72	45	1.66 ± 0.39	1.13 ± 0.55	2.78 ± 0.26A	2.49 ± 0.20A
	50	4.18 ± 0.23	1.13 ± 0.55	5.31 ± 0.12A	5.04 ± 0.21A

[a] Mean values ± standard deviation. Means with the same upper-case letter in the same row are not significantly different ($P > 0.05$).

Table IV-5. Comparison of reductions of *S.* Typhimurium in apple juice by heat or ozone alone and the combination treatment.

°Brix	Treatment temperature (°C)	Heat	Ozone (at 25°C)	Sum	Combination
12	45	0.62 ± 0.42[a]	1.84 ± 0.74	2.46 ± 0.32A	2.20 ± 0.29A
	50	2.50 ± 0.40	1.84 ± 0.74	4.34 ± 0.29B	5.30 ± 0.08A
18	45	1.40 ± 0.11	1.38 ± 0.45	2.78 ± 0.34A	2.64 ± 0.25A
	50	3.95 ± 0.20	1.38 ± 0.45	5.33 ± 0.33A	5.03 ± 0.20A
36	45	1.60 ± 0.47	0.74 ± 0.35	2.34 ± 0.23A	2.83 ± 0.59A
	50	3.03 ± 0.27	0.74 ± 0.35	3.77 ± 0.43B	4.56 ± 0.08A
72	45	2.09 ± 0.31	1.17 ± 0.46	3.26 ± 0.29A	2.80 ± 0.46A
	50	4.11 ± 0.41	1.17 ± 0.46	5.28 ± 0.45A	5.08 ± 0.24A

[a] Mean values ± standard deviation. Means with the same upper-case letter in the same row are not significantly different ($P > 0.05$).

Generally, food scientists used to believe that juice was safe from foodborne pathogens due to the low pH of juice. But, unfortunately, there are several studies which confirm the survival of pathogens in juice (Ryu and Beuchat, 1998; Oyarzábal et al., 2003). Pathogens can survival in acidic fruit juice because of acid adaptation. *E. coli* O157:H7 and *S.* Typhimurium cells can develop adaptive mechanisms by undergoing genetic and physiologic changes that allow the cells to stay viable in acidic juice (Foster and Hall, 1990; Lin et al., 1996). Acid adaption of pathogens shows cross-protection against thermal treatment (Ryu and Beuchat, 1998). In actuality, the presence of pathogens in acidic juice triggers the acid adaptation phenomenon, which can produce a different response to treatment. In this study, I did not confirm the effect of acid adaptation of pathogens on combined treatment of heat and ozone. But Gabriel (2012) reported that the effect of physicochemical combination on decimal reduction times showed that acid-adapted *E. coli* O157:H7 had no cross-protection effect against thermal treatment in liquid medium (pH 3.0, 55 °Brix at 55°C). In this respect, I can postulate that acid-adapted pathogens in apple juice with high

soluble solids content do not show any cross-protection effect.

Ozone has a high oxidation potential, so it can be used as a decolorizing agent. Ozone oxidizes or breaks -C=C- bonds, the -N=N- bonds, and heterocyclic and aromatic rings (Strickland and Perkins, 1995). These lead to color changes of food during ozone treatment. During ozone treatment, color changes of blackberry juice (Tiwari et al., 2009a), grape juice (Tiwari et al., 2009c), apple juice (Torres et al., 2011) and orange juice (Tiwari et al., 2008) were reported. However, in this study, no significant changes of *L*, *a*, and *b* values were observed in apple juice. Color changes of fruit juices by ozone treatment can be affected by parameters such as treatment time, concentration of ozone, and flow rate of ozone. Particularly, in this study, treatment time was shorter than that of other investigations.

According to the Code of Federal Regulations, the maximum residual ozone level is 0.4 mg/L when water is bottled (FDA, 2012). However, there are no official regulations regarding a measurement method for residual ozone in liquid foods such as apple juice. This may be due to the rapid decomposition property of ozone. Therefore, I analyzed residual ozone by

the indigo method which is commonly used to determine concentration of ozone in water. After ozone treatment, residual ozone concentration in all samples decreased to below 0.4 mg/L when maintained at 4°C for 6 h. I also confirmed that residual ozone increased as the treatment temperature decreased, correlating with the result of a previous study (Archen et al., 2001). They reported that the concentration of residual ozone was greatest at 4°C, not at 22 or 45°C.

It has been reported that ozone treatment is more effective when microorganisms are suspended in pure water or buffers containing less ozone demanding materials than in complex food systems composed of organic compounds (Cho et al., 2003). Ozone concentration in treated apple juice couldn't be determined by the indigo method (data not shown) because of intrinsic color of juice, but residual ozone in distilled water could be analyzed. Even though I obtained results about residual ozone by using distilled water instead of apple juice, low concentrations of residual ozone may occur in apple juice due to various ozone consuming compounds compared to distilled water. There are organic compounds such as sugars,

pectic substances, and antioxidant compounds in apple cider and orange juice. These compounds may react with ozone (Kim et al., 1999; Williams et al., 2004). Antioxidant compounds like polyphenols, phenolic acids, flavonoids and ascorbic acid are ozone consuming materials having an effect on ozone chelation in apple juice (Liao et al., 2007). Therefore, application of the indigo method in this study is considered reasonable for judging the concentration of residual ozone in apple juice by inference through results obtained from distilled water.

In conclusion, this study validates the possibility of the combination of ozone and heat treatment as a novel technology for inactivating foodborne pathogens in apple juice of different solids content (°Brix). I found the combination effect and, in some cases the synergistic effect, when apple juice was treated with ozone and heat. Also, following treatment, there were no significant differences in color values. Also, the concentration of residual ozone in juice might decrease to below 0.4 mg/L after ozone treatment. If this intervention is to be used in the food industry, processing for more rapid ozone decomposition is necessary and specific treatment conditions such as

temperature, time and ozone concentration should be established considering inactivation of pathogens and maintenance of sensory quality in apple juice.

IV-2. Inactivation of foodborne pathogens on seeds by combining vacuumed hydrogen peroxide vapor and vacuum-sealed dry heat

IV-2.1. Introduction

Seed sprouts have gained much attention due to the demand for nutritious, healthful foods. And also, many types of seed sprouts are a part of traditional east Asian cuisine. But often, seed sprouts (especially alfalfa sprouts) are consumed raw in Europe or America which means that sprouts can carry foodborne pathogens and cause foodborne illnesses (Hong and Kang, 2016) as I mentioned in Chapter I.

Because of these reasons many researchers have studied pasteurization methods for seeds. Mazzoni et al. (2001) reported that 4,000 psi supercritical carbon dioxide for 60 min reduced *E. coli* K12 on alfalfa seeds by about 1.0 log CFU/g. Sharma et al. (2002) used ozonated water to reduce *E. coli* O157:H7 on alfalfa seeds. Twenty-one ppm ozone treatment for 64 min resulted in 1.49 log reduction of this pathogen. Electrolyzed oxidizing water treatment (concentration of chlorine was 84 mg/l) for 10 min reduced *Salmonella* on alfalfa seeds by 1.65 log CFU/g (Kim et al., 2003). These non-thermal treatments did not achieve 5 log reductions which is

recommended by the US FDA. Additionally, pulsed UV-light (Sharma and Demirci, 2003), ultrasound, irradiation, acidic electrolyzed water (Kim et al., 2006) and combinations of chemical, heat and ultrasound (Scouten and Beuchat, 2002) were also used to pasteurize alfalfa seeds but these treatments also did not reduce pathogens by more than 5 log CFU/g, or, they adversely affected seed germinability.

Hydrogen peroxide is a GRAS (generally recognized as safe) compound widely investigated for inactivating microorganisms in foods. The main target of hydrogen peroxide is the cell membrane because hydrogen peroxide forms the hydroxyl radical (•OH) (McDonnell and Russell, 1999). But several studies have confirmed that aqueous hydrogen peroxide does not effectively reduce pathogen populations on food surfacces. Hong and Kang (2016) reported that 2% hydrogen peroxide treatment for 10 min reduced *Salmonella* Typhimurium by 1.41 log CFU/g. Svoboda et al. (2016) used 5% hydrogen peroxide treatment to reduce pathogens on the surface of melons. This 2 min treatment reduced *E. coli* O157:H7 and *Salmonella* by about 1.5 log CFU/g. But hydrogen peroxide vapor (HPV) is more effective

than aqueous hydrogen peroxide at inactivating pathogens on food surfaces. Back et al. (2014) reported that 10% HPV for 10 min reduced levels of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* on lettuce by about 3 log CFU/g without incurring quality deterioration.

Recently, Hong (2016) used the combination treatment of aqueous hydrogen peroxide and vacuum-sealed dry heat to reduce pathogens on alfalfa seeds. But a long time interval is required for dehydration after aqueous hydrogen peroxide treatment and also dry heat treatment. But, to date, there have been no studies documenting inactivation of pathogens on seeds using the combination treatment of HPV and vacuum-sealed dry heat. Recently, Plasmapp co., Ltd. (Daejeon, Republic of Korea) developed a vacuumed hydrogen peroxide vapor sterilization device (STERLINK®, model: FPS-15s) for medical use. I also confirmed that vacuumed hydrogen peroxide vapor treatment effectively reduced pathogen levels in black and red pepper (Chapter II-2). Therefore, the objectives of this study were to evaluate efficacy of the combination treatment of vacuumed hydrogen peroxide vapor and vacuum-sealed dry heat to inactivate *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* on alfalfa seeds and document its effect on seed germination rate.

IV-2.2. Materials and Methods

Bacterial strains and cell suspension Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19586, ATCC 43174, ATCC 700408) and *L. monocytogenes* (ATCC 19111, ATCC 19114, ATCC 19115) were used. Each strain was obtained from the bacteria culture collection of Seoul National University (Seoul, Korea) for this study. Stock cultures were prepared by mixing 0.7 ml of a tryptic soy broth (TSB; Difco, BD, Sparks, MD) 24 h, 37°C culture with 0.3 ml of sterile 50% glycerol and then storing at –80°C. Working cultures were streaked onto tryptic soy agar (TSA; Difco, BD), incubated at 37°C for 24 h and stored at 4°C.

Preparation of inocula For each experiment, inoculum was prepared individually for each strain using the method described by Hong and Kang (2016). A single colony of each strain was cultured in 5 ml of TSB at 37°C for 24 h. For production of a bacterial lawn, 1 ml of the overnight culture

was spread onto each of 5 TSA plates followed by incubation at 37°C for 24 h. The bacterial lawn was dislodged by adding 4.5 ml of 0.2% peptone water (PW; Difco) to each plate and rubbing with a sterile cotton swab. Cell suspensions were collected from the five plates and pooled, corresponding to approximately 10^{12} CFU/ml. This pooled cell suspension was diluted with 0.2% PW to yield 500 ml of culture cocktail (9 strains total).

Sample preparation and inoculation Alfalfa seeds were purchased from a local seed retail company (Danong, Namyangju, Republic of Korea). For inoculation, 500 g samples were immersed in 500 ml of prepared inoculum and then gently agitated in this mixture for 5 min. The inoculated samples were dried for 24 h inside a biosafety hood (25°C) with the fan running until the moisture content of the samples equaled that of a non-inoculated sample (a_w 0.22). The final cell concentration was 7 to 8 log CFU/g. Moisture content of alfalfa seeds was measured with a Mettler-Toledo HB43-S moisture analyzer (Mettler-Toledo, Columbus, OH).

Vacuumed hydrogen peroxide vapor treatment Vacuumed hydrogen peroxide vapor treatment was performed with the same apparatus used in chapter III-2 (Fig. III-1), which was recently developed for vacuum sterilization processing. Alfalfa seed samples in petri dishes were placed on a 2 cm high plastic rack to avoid heat from the chamber surface and the cover was closed. A initial pumping process commenced until atmospheric pressure of chamber was less than 1.5 torr. Chamber and needle valves were opened and 30% hydrogen peroxide (1.0 ml) was injected through the injection port by syringe. Sterile distilled water was used as a control. Injected hydrogen peroxide or distilled water was vaporized with a 220°C heater and dispersed into the chamber. After injection, chamber vacuum was maintained for 1 min to allow for diffusion of HPV. After 1 min, chamber and needle valves were closed. A second pumping step was then initiated for removal of HPV in the treatment chamber until 1.5 torr was attained. Then the vacuum pump was turned off and the venting valve was opened. The chamber cover was opened after chamber and atmospheric pressure equilibrated then samples were collected.

Dry heat and vacuum-sealed dry heat treatment For dry heat treatment, 10 g of vacuumed hydrogen peroxide vapor-treated or non-treated alfalfa seeds were placed in a 90 mm petri dish and the cover was removed during heat treatment. For the vacuum-sealed dry heat treatment, 10 g of vacuumed hydrogen peroxide vapor-treated or non-treated alfalfa seeds were placed in aseptic nylon-polyethylene bags and vacuum-sealed with a packaging machine (Airzero, Ansan, Republic of Korea). The samples were then moved to an oven (Thermostable^{TM} ON-32, Wisd laboratory instruments) with a temperature of 73°C and heated up for up to 2 h in 30 min time increments.

Bacterial enumeration Collected samples (10 g) were diluted in 90 ml of 0.2% PW and homogenized for 2 min in a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1 ml aliquots of homogenized samples were tenfold serially diluted in 9 ml of 0.2% PW, and 0.1 ml of sample or diluent was spread-plated onto each selective medium. Sorbitol MacConkey agar (Difco), Xylose Lysine Desoxycholate agar

(Difco) and Oxford agar base with antimicrobial agent (Difco) were used as selective media for the enumeration of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes*, respectively. Where low populations of surviving cells were anticipated, 1 ml aliquots of the original homogenate were equally distributed onto four plates of each respective medium and spread-plated. All plates were incubated at 37°C for 24 h and colonies were counted.

Effect of the combination treatment of vacuumed hydrogen peroxide vapor and vacuum-sealed dry heat on viability of alfalfa seeds Two hundred randomly selected treated or control seeds were placed on sterile cheesecloth in 90 mm diameter petri dishes, and periodically provided with sufficient distilled water to foster seed germination. The seeds were incubated at room temperature (21°C) for 5 days. Only seeds with a hypocotyl protruding were counted as a sprout. Ruptured or swollen seeds were not counted. Experiments were performed in triplicate.

Statistical analysis All data were analyzed with one-way ANOVA using

the Statistical Analysis System (SAS Institute, Cary, NC, USA) and Duncan`s multiple range test to determine if there were significant differences ($P < 0.05$) in mean values of microorganism populations. Microbial counts were transformed to log values for analysis. One log was used for calculations in the case of populations below the detection limit.

IV-2.3. Results

Initial populations of pathogens on inoculated alfalfa seeds were approximately 10^6-10^7 CFU/g. In uninoculated samples pathogen numberss were below the detection limit (1.0 log CFU/g). The combination treatment of vacuumed hydrogen peroxide vapor + vacuum-sealed dry heat greatly reduced levels of pathogens on alfalfa seeds.

The inactivation effects of the combination of vacuumed hydrogen peroxide vapor and dry heat or vacuum-sealed dry heat on *E. coli* O157:H7 on alfalfa seeds are shown in Table IV-6. The initial population of *E. coli* O157:H7 on alfalfa seeds was 7.52 log CFU/g and when alfalfa seeds were subjected to 0 and 30% vacuumed hydrogen peroxide vapor, populations on seeds were reduced to 7.41 and 6.54 log CFU/g, respectively. *E. coli* O157:H7 populations on alfalfa seeds sequentially treated with vacuumed hydrogen peroxide vapor (30%) and vacuum-sealed dry heat (73°C) for 2 h decreased to below the detection limit (1.0 log CFU/g). With dry heat treatment, the control, 0% vacuumed hydrogen peroxide vapor treated and

30% vacuumed hydrogen peroxide vapor treated alfalfa seeds yielded a maximum of 1.42, 2.06 and 2.62 log CFU/g reductions of this pathogen, respectively. Considering vacuum-sealed dry heat treatment, the control, 0% vacuumed hydrogen peroxide vapor treated and 30% vacuumed hydrogen peroxide vapor treated alfalfa seeds resulted in 4.77, 6.12 and > 6.52 log CFU/g reductions, respectively, of *E. coli* O157:H7.

Table IV-7 shows the bactericidal effect of the combination of vacuumed hydrogen peroxide vapor and dry heat or vacuum-sealed dry heat against *S.* Typhimurium on alfalfa seeds. The initial population of *S.* Typhimurium on alfalfa seeds was 7.54 log CFU/g and when alfalfa seeds were subjected to 0 and 30% vacuumed hydrogen peroxide vapor, populations on seeds were reduced to 7.40 and 6.64 log CFU/g, respectively. *S.* Typhimurium populations on alfalfa seeds sequentially treated with vacuumed hydrogen peroxide vapor (30%) and vacuum-sealed dry heat (73°C) for 2 h decreased to below the detection limit (1.0 log CFU/g). With dry heat treatment, the control, 0% vacuumed hydrogen peroxide vapor treated and 30% vacuumed hydrogen peroxide vapor treated alfalfa seeds

yielded a maximum of 1.28, 3.39 and 2.75 log CFU/g reductions of this pathogen, respectively. Regarding vacuum-sealed dry heat treatment, the control, 0% vacuumed hydrogen peroxide vapor treated and 30% vacuumed hydrogen peroxide vapor treated alfalfa seeds experienced 5.32, 6.14 and > 6.54 log CFU/g reductions of *S.* Typhimurium, respectively.

The inactivation effects of vacuumed hydrogen peroxide vapor combined with dry heat or vacuum-sealed dry heat on *L. monocytogenes* on alfalfa seeds are shown in Table IV-8. The initial population of *L. monocytogenes* on alfalfa seeds was 7.34 log CFU/g and when alfalfa seeds were subjected to 0 and 30% vacuumed hydrogen peroxide vapor, cell counts were reduced to 7.04 and 6.48 log CFU/g, respectively. *L. monocytogenes* populations on alfalfa seeds sequentially treated with vacuumed hydrogen peroxide vapor (30%) and vacuum-sealed dry heat (73°C) for 2 h were decreased to below the detection limit (1.0 log CFU/g). With dry heat treatment, the control, 0% vacuumed hydrogen peroxide vapor and 30% vacuumed hydrogen peroxide vapor treated alfalfa seeds yielded a maximum of 2.48, 2.09 and 2.83 log CFU/g reductions,

respectively, of this pathogen. With regard to vacuum-sealed dry heat treatment, the control, 0% vacuumed hydrogen peroxide vapor and 30% vacuumed hydrogen peroxide vapor treated alfalfa seeds resulted in 4.69, 5.94 and > 6.34 log CFU/g reductions of *L. monocytogenes*, respectively.

Table IV-6. Population of *E. coli* O157:H7 on alfalfa seeds treated with sequential treatments.

Treatment	Dry heat time (min)				
	0	30	60	90	120
	Populations (log CFU/g)				
Dry heat	7.52 ± 0.48[a] Aa	6.72 ± 0.22 Ab	6.29 ± 0.26 Abc	6.23 ± 0.20 Abc	6.10 ± 0.26 Ac
Vacuum-sealed dry heat	7.52 ± 0.48 Aa	6.35 ± 0.53 Ab	4.30 ± 0.52 CDc	3.46 ± 0.45 Cd	2.75 ± 0.13 Dd
DW + Dry heat	7.41 ± 0.30 Aa	6.39 ± 0.36 Ab	5.92 ± 0.37 Abc	5.71 ± 0.38 Ac	5.46 ± 0.15 Bd
DW + Vacuum-sealed dry heat	7.41 ± 0.30 Aa	5.52 ± 0.24 Bb	5.05 ± 0.31 Bb	3.38 ± 0.54 Cc	1.40 ± 0.35 Ed
H_2O_2 + Dry heat	6.54 ± 0.39 Ba	5.09 ± 0.15 Bb	4.73 ± 0.26 BCb	4.79 ± 0.44 Bb	4.90 ± 0.17 Cb
H_2O_2 + Vacuum-sealed dry heat	6.54 ± 0.39 Ba	4.85 ± 0.49 Bb	4.01 ± 0.41 Dc	1.83 ± 0.30 Dd	< 1.00 ± 0.00 Fe

[a] Mean values ± standard deviation. Means followed by the same uppercase letters within a row and by the same lowercase letters within a column are not significantly different ($P< 0.05$).

Table IV-7. Population of *S.* Typhimurium on alfalfa seeds treated with sequential treatments.

Treatment	Dry heat time (min)				
	0	30	60	90	120
	Populations (log CFU/g)				
Dry heat	7.54 ± 0.30[a] Aa	6.53 ± 0.21 Ab	6.40 ± 0.10 Ab	6.26 ± 0.38 Ab	6.26 ± 0.24 Ab
Vacuum-sealed dry heat	7.54 ± 0.30 Aa	6.17 ± 0.23 Ab	4.43 ± 0.52 Cc	2.74 ± 0.52 Cd	2.23 ± 0.29 Dd
DW + Dry heat	7.40 ± 0.30 Aa	6.16 ± 0.25 Ab	5.67 ± 0.35 Bbc	5.53 ± 0.44 Ac	4.16 ± 0.28 Cd
DW + Vacuum-sealed dry heat	7.40 ± 0.30 Aa	5.34 ± 0.23 Bb	4.46 ± 0.58 Cc	2.63 ± 0.35 Cd	1.40 ± 0.35 Ee
H_2O_2 + Dry heat	6.64 ± 0.28 Ba	5.07 ± 0.16 BCb	4.60 ± 0.30 Cb	4.50 ± 0.51 Bb	4.79 ± 0.19 Bb
H_2O_2 + Vacuum-sealed dry heat	6.64 ± 0.28 Ba	4.56 ± 0.54 Cb	3.87 ± 0.40 Cc	1.86 ± 0.31 Dd	< 1.00 ± 0.00 Ee

[a] Mean values ± standard deviation. Means followed by the same uppercase letters within a row and by the same lowercase letters within a column are not significantly different ($P< 0.05$).

Table IV-8. Population of *L. monocytogenes* on alfalfa seeds treated with sequential treatments.

Treatment	Dry heat time (min)				
	0	30	60	90	120
	Populations (log CFU/g)				
Dry heat	7.34 ± 0.30[a] Aa	6.36 ± 0.39 Ab	5.55 ± 0.39 ABc	5.17 ± 0.13 Acd	4.87 ± 0.38 ABd
Vacuum-sealed dry heat	7.34 ± 0.30 Aa	6.85 ± 0.22 Aa	5.96 ± 0.29 Ab	3.75 ± 0.53 Bc	2.65 ± 0.56 Cd
DW + Dry heat	7.04 ± 0.51 ABa	5.36 ± 0.39 BCb	5.26 ± 0.24 BCb	4.92 ± 0.13 Ab	5.25 ± 0.05 Ab
DW + Vacuum-sealed dry heat	7.04 ± 0.51 ABa	5.16 ± 0.22 BCb	5.45 ± 0.39 ABb	2.30 ± 0.52 Cc	1.40 ± 0.35 Dd
H_2O_2 + Dry heat	6.48 ± 0.30 Ba	5.47 ± 0.38 Bb	4.84 ± 0.18 Cc	4.59 ± 0.27 A	4.52 ± 0.40 Bd
H_2O_2 + Vacuum-sealed dry heat	6.48 ± 0.30 Ba	4.73 ± 0.38 Cb	4.19 ± 0.20 Dc	1.53 ± 0.40 Dd	< 1.00 ± 0.00 Dd

[a] Mean values ± standard deviation. Means followed by the same uppercase letters within a row and by the same lowercase letters within a column are not significantly different ($P< 0.05$).

Fig. IV-10 shows the germination rate of control, 0 or 30% vacuumed hydrogen peroxide vapor treated alfalfa seeds in combination with 2 h vacuum-sealed dry treatment. The germination rate of seeds subjected to this combination treatment was not significantly different ($P > 0.05$) from that of the control. And there was no significant difference between 0% and 30% vacuumed hydrogen peroxide vapor treated sample after subjection to vacuum-sealed dry heat for 2 h

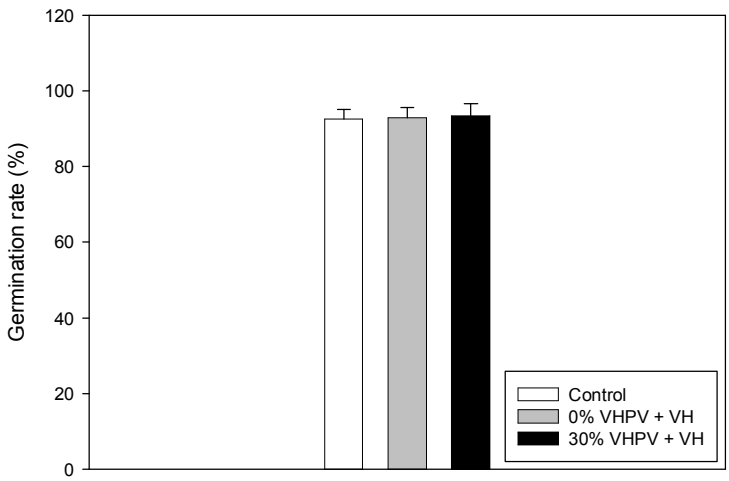


Figure IV-10. Germination rate of alfalfa seeds after sequential treatment. Alfalfa seeds control (white bar), treated with 1.0 ml 0% vacuumed hydrogen peroxide vapor (VHPV) (gray bar) or 1.0 ml of 30% vacuumed hydrogen peroxide vapor (VHPV) (black bar) followed by vacuum-sealed dry heat (VH) at 73°C for 2 h. No significant difference was observed ($P > 0.05$).

IV-2.4. Discussion

A number of studies have reported difficulties in attempting to pasteurize seeds by means of chemicals (Sharma et al., 2002; Kim et al., 2003; Scouten and Beuchat, 2002). This is due to cracks and crevices harboring pathogens on seed surfaces where chemicals can not contact them (Taormina and Beuchat, 1999). Therefore, many researchers have focused their attention on developing techniques to reduce seedborne human pathogens. Dry heat is one of those treatments. But usually, dry heat treatment requires excessive time to reduce pathogens by 5 log. Feng et al. (2007) reported that dry heat at 55°C for 4 days reduced *E. coli* O157:H7 and *Salmonella* on alfalfa seeds by more than 5 log CFU/g. Neetoo and Chen (2011) reported that dry heat treatment at 60°C for 10 days reduced *Salmonella* on alfalfa seeds by only 2.1 log CFU/g. To eliminate the long treatment times needed for dry heat treatment, investigators have experimented with combining chemicals and dry heat. Hong and Kang (2016) reported that sequential treatment with dry heat (60, 70 and 80°C)

and 2% hydrogen peroxide resulted in 1.46, 2.83 and 3.60 log reductions of *S.* Typhimurium, respectively, after 24 h treatment. Bang et al. (2011a) reported that dry heat at 55°C for 12 h following 5 min of 500 ppm chlorine dioxide treatment and drying for 2 h at 25°C reduced *E. coli* O157:H7 on radish seeds to under the detection limit (0.8 log CFU/g). Also, Bang et al (2011b) reported that 500 ppm chlorine dioxide treatment for 5 min and drying for 24 h at 45°C reduced *E. coli* O157:H7 on radish seeds to under the detection limit (1.0 log CFU/g).

Hong (2016) used the sequential treatment of hydrogen peroxide and dry heat to reduce *S.* Typhimurium on alfalfa seeds. This sequential treatment effectively reduced this pathogen but took a long time. After 10 min of hydrogen peroxide exposure, drained seeds were dried for 14 h and treated with dry heat at 73°C for 4 h to reduce *S.* Typhimurium to under the detection limit. In total, this sequential treatment takes 18 h and 10 min. I reduced the total treatment time to less than 2 h and 30 min. The main reason for this great reduction in treatment time is vacuumed hydrogen peroxide vapor treatment. When using aqueous hydrogen peroxide treatment,

drying is required. Seeds are marketed in dry condition and also the germinability of wet seeds is degraded by heat treatment. Jaquette et al. (1996) reported that hot water treatment (over 54°C) reduced the germination rate of alfalfa seeds. But vacuumed hydrogen peroxide vapor treatment does not need separate drying step. The moisture content of alfalfa seeds treated with vacuumed hydrogen peroxide vapor slightly increased, but there was no significant difference between treated and control seeds (data not shown). Also using the vapor phase of hydrogen peroxide in under vacuum has the advantage of greater diffusion into crevices or cracks in food surfaces. The gaseous phase of other chemicals has been shown to penetrate deeper into food surfaces and vacuum treatment can also help these chemicals penetrate into crevices and cracks (Kang and Kang, 2016).

Hydrogen peroxide is primarily a bleaching agent known to cause color changes to foods. There have been several studies which demonstrate color or quality changes during hydrogen peroxide treatment. Alexandre et al. (2012) and McWatters et al. (2002) reported that hydrogen peroxide treatment adversely affected quality of strawberries, watercress, red bell

peppers and lettuce. But the main quality attribute of seeds is germination rate. Several studies have documented the effect of hydrogen peroxide on seed germination. Ishibashi et al. (2008) reported that hydrogen peroxide induced the germination of wheat seeds and Barba-Espin (2010) reported that hydrogen peroxide induced pea seed germination. But, in this study, vacuumed hydrogen peroxide vapor treatment on alfalfa seeds did not induce seed germination. Hong and Kang (2016) also reported that hydrogen peroxide treatment did not induce seed germination. The germination rates of control, DW treated and hydrogen peroxide treated samples were not significantly different. Hong and Kang (2016) reported that residual hydrogen peroxide levels of seeds subjected to 2% hydrogen peroxide treatment for 10 min were reduced to under the detection limit after 24 h. Back et al. (2014) also reported that residual hydrogen peroxide in leachate from lettuce treated with 10% HPV for 10 min was reduced to under the detection limit after 36 h storage at 4°C. In the present study, I did not measured residual hydrogen peroxide in foods but the total volume of

hydrogen peroxide (30%, 1 ml) used in this study was smaller than that of Back et al. (2014).

In conclusion, I can recommend the combination of vacuumed hydrogen peroxide vapor + vacuum-sealed dry heat as a pasteurization intervention for seeds. This combination treatment reduced *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* on alfalfa seeds by > 6.52, > 6.54 and > 6.34 log CFU/g, respectively, while not affecting germination rate. Further investigation is needed to quantify the residual hydrogen peroxide after combination treatment.

References

Achen M, Yousef AE. 2001. Efficacy of ozone against *Escherichia coli* O157:H7 on apples. Journal of food science **66**: 1380-1384.

Aguilar-Rosas SF, Ballinas-Casarrubias ML, Nevarez-Moorillon GV, Martin-Belloso O, Ortega-Rivas E. 2007. Thermal and pulsed electric fields pasteurization of apple juice: Effect on physicochemical properties and flavour compounds. Journal of Food Engineering **83**: 41-46.

Ahn DU, Nam KC, Du M, Jo C. 2001. Effect of irradiation and packaging conditions after cooking on the formation of cholesterol and lipid oxidation products in meats during storage. Meat Science **57**: 413-418.

Akbas MY, Ozdemir M. 2008. Effect of gaseous ozone on microbial inactivation and sensory of flaked red peppers. International Journal of Food Science and Technology 43: 1657-1662.

Alakomi, HL, Skyttä E, Saarela M, Mattila-Sandholm T, Latva-kala K, Helander IM. 2000. Lactic Acid Permeabilizes Gram-Negative Bacteria by Disrupting the Outer Membrane. Applied and Environmental

Microbiology 66: 2001-2005.

Alberto I, Martín-Diana AB, Cullen PJ, Tiwari BK, Ojha SK, Bourke P, Álvarez C, Rico D. 2017. Effect of dielectric barrier discharge (DBD) generated plasma on microbial reduction and quality parameters of fresh mackerel (*Scomber scombrus*) fillets. Innovative Food Science and Emerging Technologies. http://dx.doi.org/10.1016/j.ifset.2017.07.006

Alexandre EMC, Brandão TRS, Silva CLM. 2012. Assessment of the impact of hydrogen peroxide solution on microbial loads and quality of red bell pepper, strawberries and watercress. Food Control 27: 362-368.

Almond Board of California. 2007a. Considerations for Proprietary Processes Used for Almond Pasteurization and Treatment. Available at: http://www.almonds.com/sites/default/files/content/attachments/proprietary-processes.pdf

Almond Board of California. 2007b. Guidelines for Validation of Dry Roasting Processes. Available at : http://www.almonds.com/sites/default/files/content/attachments/dry-roast-validation-guidelines.pdf

Álvarez-Ordóñez A, Fernández A, Bernardo A, López M. 2009. A Comparative Study of Thermal and Acid Inactivation Kinetics in Fruit Juices of *Salmonella enterica* Serovar Typhimurium and *Salmonella enterica* Serovar Senftenberg Grown at Acidic Conditions. Foodborne Pathogens and Disease 6: 1147-1155.

American Oil Chemists' Society. 1998. Official Methods and Recommended Practices of the AOCS, 5th ed. AOCS Press, Champaign, IL (USA).

American Oil Chemists' Society. 2009. Official Methods and Recommended Practices of the AOCS, 6th ed. AOCS Press, Champaign, IL (USA).

Armbruster G, Haefele C. 1975. Quality of foods after cooking in 915 MHz and 2450 MHz microwave appliances using plastic film covers. Journal of Food Science 40: 721-723.

Back KH, Ha JW, Kang DH. 2014. Effect of hydrogen peroxide vapor treatment for inactivating *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* on organic fresh lettuce. Food

Control 44: 78-85.

Bader H, Hoigni J. 1981. Determination of ozone in water by the indigo method. Water Research 15: 449-456.

Baltes W. 1982. Chemical Changes in Food by the Maillard Reaction. Food Chemistry 9: 59-73.

Ban GH, Kang DH. 2014. Effects of gamma irradiation for inactivating *Salmonella* Typhimurium in peanut butter during storage. International Journal of Food Microbiology 171: 48-53.

Ban GH, Kang DH. 2016. Effect of sanitizer combined with steam heating on the inactivation of foodborne pathogens in a biofilm on stainless steel. Food Microbiology 55: 47-54.

Bang J, Kim H, Kim H, Beuchat LR, Ryu JH. 2011a. Combined effects of chlorine dioxide, drying, and dry heat treatments in inactivating microorganisms on radish seeds. Food Microbiology 28: 114-118.

Bang J, Kim H, Kim H, Beuchat LR, Ryu JH. 2011b. Inactivation of *Escherichia coli* O157:H7 on Radish Seeds by Sequential Treatments with Chlorine Dioxide, Drying, and Dry Heat without Loss of Seed

Viability. Applied and Environmental Microbiology 77: 6680-6686.

Barba-Espin G, Diaz-Vivancos P, Clemente-Moreno MJ, Albacete A, Faize L, Faize M, Pérez-Alfocea F, Hernández JA. 2010. Interaction between hydrogen peroxide and plant hormones during germination and the early growth of pea seedlings. Plant, Cell and Environment 33: 981-994.

Bari ML, Nei D, Sotome I, Nishina I, Isobe S, Kawamoto S. 2009. Effectiveness of Sanitizers, Dry Heat, Hot Water, and Gas Catalytic Infrared Heat Treatments to Inactivate Salmonella on Almonds. Foodborne Pathogens and Disease 6: 953-958.

Bayındırlı A, Alpas H, Bozoğlu F, Hızal M. 2006. Efficiency of high pressure treatment on inactivation of pathogenic microorganisms and enzymes in apple, orange, apricot and sour cherry juices. Food Control 17: 52-58.

Besser RE, Griffin PM, Slutsker L. 1999. *Escherichia coli* O157:H7 Gastroenteritis and the Hemolytic Uremic Syndrome: An Emerging Infectious Disease. Annual Reviews of Medicine 50: 355-367.

Beuchat LR, Brackett RE, Hao DYY, Conner, DE. 1986. Growth and thermal inactivation of *Listeria monocytogenes* in cabbage and cabbage juice. Canadian Journal of Microbiology 32: 791-795.

Beuchat LR, Mann DA. 2011. Inactivation of *Salmonella* on Pecan Nutmeats by Hot Air Treatment and Oil Roasting. Journal of Food Protection 74: 1441-1450.

Bharate SS, Bharate SB. 2014. Non-enzymatic browning in citrus juice: chemical markers, their detection and ways to improve product quality. Journal of Food Science and Technology 51: 2271-2288.

Bigelow WD, Esty JR. 1920. The thermal death point in relation to typical thermophilic organisms. Journal of Infectious Diseases 27: 602.

Bignon J. 1997. Cold pasteurizers hyperbar for the stabilization of fresh fruit juices. Fruit processing 6: 46–48.

Blessington T, Mitcham EJ, Harris LJ. 2012. Survival of *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on Inoculated Walnut Kernels during Storage. Journal of Food Protection 75: 245-254.

Bolling BW, Chen CYO, McKay DL, Blumberg B. 2011. Tree nut phytochemicals: composition, antioxidant capacity, bioactivity, impact factors. A systemic review of almonds, Brazils, cashews, hazelnuts, macadamias, pecans, pine nuts, pistachios and walnuts. Nutrition Research Review 24: 244-275.

Boyer J, Liu RH. 2004. Apple phytochemicals and their health benefits. Nutrition Journal 3: 5.

Boyle EC, Bishop JL, Grassl GA, Finlay BB. 2007. *Salmonella*: from Pathogenesis to Therapeutics. Journal of Bacteriology 189: 1489-1495.

Braddock RJ. 1999. Single strength orange juices and concentrate. In handbook of citrus by-products and processing technology. Wiley, New York

Brandl MT, Pan, Z, Huynh, S, Zhu Y, McHugh TH. 2008. Reduction of *Salmonella* Enteritidis Population Sizes on Almond Kernels with Infrared Heat. Journal of Food Protection 71: 897-902.

Brockmann SO, Piechotowski I, Kimmig P. 2004. *Salmonella* in Sesame Seed Products. Journal of Food Protection 67: 178-180.

Buchanan RL, Edelson SG, Snipes K, Boyd G. 1998. Inactivation of *Escherichia coli* O157:H7 in apple juice by irradiation. Applied and Environmental Microbiology 64: 4533-4535.

Buckenhüskes HJ, Rendlen M. 2004. Hygienic Problems of Phytogenic Raw Materials for Food Production with Special Emphasis to Herbs and Spices. Food Science and Biotechnology 13: 262-268.

Burleson GR, Murray TM, Pollard M. 1975. Inactivation of viruses and bacteria by ozone with and without sonication. Applied Microbiology 29: 340–344.

Burnett SL, Gehm ER, Weissinger WR, Beuchat LR. 2000. Survival of *Salmonella* in peanut butter and peanut butter spread. Journal of Applied Microbiology 89: 472-477.

Burow H, Pudich U. 1996. Different *Salmonella* serovars in spices, flavoured potato crisps and other food. Fleischirtschaft 76: 640-644.

Caminiti IM, Palgan I, Muñoz A, Noci F, Whyte P, Morgan DJ, Cronin DA, Lyng JG. 2012. The Effect of Ultraviolet Light on Microbial Inactivation and Quality Attributes of Apple Juice. Food and Bioprocess

Technology 5: 680-686.

Cañumir JA, Celis JE, de Bruijn J, Vidal LV. 2002. Pasteurisation of Apple Juice by Using Microwaves. LWT-Food Science and Technology 35: 389-392.

Cavallaro E, Date K, Medus C, Meyer S, Miller B, Kim C, Nowicki S, Cosgrove S, Sweat D, Phan Q, Flint J, Daly ER, Adams J, Hyytia-Trees E, Gerner-Smidt P, Hoekstra RM, Schwensohn C, Langer A, Sodha SV, Rogers MC, Angulo FJ, Tauxe RV, Williams IT, Behravesh CB. 2011. *Salmonella* Typhimurium infections associated with peanut products. The New England Journal of Medicine 365: 601-610.

Centers for Disease Control and Prevention. 1993. *Salmonella* Serotype Tennessee in Powdered Milk Products and Infant Formula – Canada and the United States. Morbidity and Mortality Weekly Report 42: 516-517.

Centers for Disease Control and Prevention. 2004. Outbreak of *Salmonella* Serotype Enteritidis Infections Associated with Raw Almonds – United States and Canada, 2003-2004. Morbidity and Mortality Weekly

Report 53: 484-487.

Center for Disease Control and Prevention. 2007a. Salmonellosis – Outbreak Investigation, February 2007. Available at : http://www.cdc.gov/ncidod/dbmd/diseaseinfo/salmonellosis_2007/030707_outbreak_notice.htm last accessed 05.01.2016.

Centers for Disease Control and Prevention. 2007b. Multistate outbreaks of *Salmonella* serotype Tennessee infections associated with peanut butter—United States, 2006-2007. Morbidity and Mortality Weekly Report 56: 521-524.

Center for Disease Control and Prevention. 2009. Multistate Outbreak of *Salmonella* Infections Linked to Pistachio Nuts (FINAL UPDATE). Available at : http://www.cdc.gov/salmonella/2009/pistachio-nuts-4-14-2009.html.

Center for Disease Control and Prevention. 2010a. Multistate Outbreak of *Salmonella* Typhimurium Infections Linked to Peanut Butter, 2008 – 2009 (Final Update). Available at : http://www.cdc.gov/salmonella/typhimurium/update.html last accessed

05.01.2016.

Center for Disease Control and Prevention. 2011a. Multistate Outbreak of *E. coli* O157:H7 Infections Associated with In-Shell Hazelnuts (Final Update). Available at : http://www.cdc.gov/ecoli/2011/hazelnuts-4-7-11.html

Centers for Disease Control and Prevention. 2011b. Multistate Outbreak of Human *Salmonella* Enteritidis Infections Linked to Alfalfa Sprouts and Spicy Sprouts (Final Update). Available at : https://www.cdc.gov/salmonella/2011/alfalfa-spicy-sprouts-7-6-2011.html

Center for Disease Control and Prevention. 2012. Multistate Outbreak of *Salmonella* Bredeney Infections Linked to Peanut Butter Manufactured by Sunland, Inc. (Final Update). Available at : http://www.cdc.gov/salmonella/bredeney-09-12/index.html

Center for Disease Control and Prevention. 2013a. Surveillance for Foodborne Disease Outbreaks – United States, 2009-2010. Morbidity and Mortality Weekly Report 62: 41-47.

Centers for Disease Control and Prevention. 2013b. Notes from the Field:

Salmonella Bredeney Infections Linked to a Brand of Peanut Butter – United States, 2012. Morbidity and Mortality Weekly Report 62: 107.

Centers for Disease Control and Prevention. 2014. Multistate Outbreak of Shiga toxin-producing *Escherichia coli* O121 Infections Linked to Raw Clover Sprouts (Final Update). Available at : https://www.cdc.gov/ecoli/2014/O121-05-14/index.html

Center for Disease Control and Prevention. 2015a. Vital Signs: Multistate Foodborne Outbreaks – United States, 2010-2014. Morbidity and Mortality Weekly Report 64: 1221-1225.

Centers for Disease Control and Prevention. 2015b. Multistate Outbreak of *Salmonella* Enteritidis Infections Linked to Bean Sprouts (Final Update). Available at : https://www.cdc.gov/salmonella/enteritidis-11-14/index.html

Centers for Disease Control and Prevention. 2015c. Wholesome Soy Products, Inc. Sprouts and Investigation of Human Listeriosis Cases (Final Update). Available at : https://www.cdc.gov/listeria/outbreaks/bean-sprouts-11-14/index.html

Centers for Disease Control and Prevention. 2016a. Foodborne Outbreak Online Database (FOOD Tool). Available at : https://wwwn.cdc.gov/foodborneoutbreaks/

Centers for Disease Control and Prevention. 2016b. Multistate Outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 Infections Linked to Alfalfa Sprouts Produced by Jack & The Green Sprouts (Final Update). Available at : https://www.cdc.gov/ecoli/2016/o157-02-16/index.html

Centers for Disease Control and Prevention. 2016c. Multistate Outbreak of *Salmonella* Infections Linked to Alfalfa Sprouts from One Contaminated Seed Lot (Final Update). Available at : https://www.cdc.gov/salmonella/muenchen-02-16/index.html

Centers for Disease Control and Prevention. 2016d. Multistate Outbreak of *Salmonella* Reading and Salmonella Abony Infections Linked to Alfalfa Sprouts (Final Update). Available at : https://www.cdc.gov/salmonella/reading-08-16/index.html

Cerf O. 1977. A review. Tailing of Survival Curves of Bacterial Spores. Journal of Applied Bacteriology 42: 1-19.

Chang SL. 1971. Modern concept of disinfection. Journal of the Sanitary Engineering Division 97: 689-707.

Chang SS, Han AR, Reyes-De-Corcuera JI, Powers JR, Kang DH. 2010. Evaluation of steam pasteurization in controlling *Salmonella* serotype Enteritidis on raw almond surfaces. Letters in Applied Microbiology 50: 393-398.

Chemat F, Zill-e-Huma, Khan MK. 2011. Applications of ultrasound in food technology: Processing, preservation and extraction. Ultrasonics Sonochemistry 18: 813-835.

Chen H, Hoover DG. 2003. Pressure inactivation kinetics of *Yersinia enterocolitica* ATCC 35669. International Journal of Food Microbiology 87: 161-171.

Cheon HL, Shin JY, Park KH, Chung MS, Kang DH. 2015. Inactivation of foodborne pathogens in powdered red pepper (*Capsicum annuum* L.) using combined UV-C irradiation and mild heat treatment. Food Control 50: 441-445.

Cho M, Chung HM, Yoon J. 2003. Disinfection of water containing

natural organic matter by using ozone-initiated radical reactions. Applied and Environmental Microbiology 69: 2284–2291.

Choi HK, Marth EH, Vasavada PC. 1993. Use of microwave energy to inactivate *Listeria monocytogenes* in milk. Milchwissenschaft 48: 200-203.

Choi MR, Liu Q, Lee SY, Jin JH, Ryu S, Kang DH. 2012. Inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in apple juice with gaseous ozone. Food Microbiology 32:191-195.

Choo E, Jang SS, Kim K, Lee KG, Heu S, Ryu S. 2007. Prevalence of Genetic Diversity of *Bacillus cereus* in Dried Red Pepper in Korea. Journal of Food Protection 70: 917-922.

Cody SH, Glynn MK, Farrar JA, Cairns KL, Griffin PM, Kobayashi J, Fyfe M, Hoffman R, King AS, Lewis JH, Swaminathan B, Bryant RG, Vugia DJ. 1999. An Outbreak of *Escherichia coli* O157:H7 Infection from Unpasteurized Commercial Apple Juice. Annals of Internal Medicine130: 202-209.

Cornucopia Institute. 2007. Fact Sheet Mandatory Sterilization of Raw Almonds. Available at : https://www.cornucopia.org/almond/Almond_Fact_Sheet.pdf

Cotter PD, Hill C. 2003. Surviving the Acid Test: Responses of Gram-Positive Bacteria to Low pH. Microbiology and Molecular Biology Reviews 67: 429-453.

Cullen PJ, Valdramidis VP, Tiwari BK, Patil S, Bourke P, O'Donnell CP. 2010. Ozone processing for food preservation: An overview on fruit Juice treatments. Ozone: Science & Engineering 32: 166–179.

Danyluk MD, Uesugi AR, Harris LJ. 2005. Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide. Journal of Food Protection 68: 1613–1622.

Danyluk MD, Jones TM, Abd SJ, .Schilitt-dittrich F, Jacobs M, Harris L. 2007. Prevalence of Amounts of Salmonella Found on Raw California Almonds. Journal of Food Protection 70: 820-827.

Das E, Gürakan GC, Bayındırlı A. 2006. Effect of controlled atmosphere storage, modified atmosphere packaging and gaseous ozone treatment on

the survival of *Salmonella* Enteritidis on cherry tomatoes. Food Microbiology 23: 430–438.

Datta AK, Davidson PM. 2000. Microwave and Radio Frequency Processing. Journal of Food Science 65: 32-41.

D'Souza T, Karwe M, Schaffner DW. 2012. Effect of High Hydrostatic Pressure and Pressure Cycling on a Pathogenic *Salmonella enterica* Serovar Cocktail Inoculated into Creamy Peanut Butter. Journal of Food Protection 75: 169-173.

Duncan SE, Moberg K, Amin KN, Wright M, Newkirk JJ, Ponder MA, Acuff GR, Dickson JS. 2017. Processes to Preserve Spice and Herb Quality and Sensory Integrity During Pathogen Inactivation. Journal of Food Science 82: 1208-1215.

Eisele TA, Drake SR. 2005. The partial compositional characteristics of apple juice from 175 apple variety. Journal of Food Composition and Analysis 18: 213-221.

El-Rawas A, Hvizdzak A, Davenport M, Beamer S, Jaczynski J, Matak K. 2012. Effect of electron beam irradiation on quality indicators of

peanut butter over a storage period. Food Chemistry 133: 212-219.

Enache E, Chen Y, Awuah G, Economides A, Scott VN. 2006. Thermal Resistance Parameters for Pathogens in White Grape Juice Concentrate. Journal of Food Protection 69: 564-569.

Evrendilek GA, Zhang QH, Richter ER. 1999. Inactivation of *Escherichia coli* O157:H7 and *Escherichia coli* 8739 in apple juice by pulsed electric fields. Journal of Food Protection 62: 793-796.

Fan X, Thayer DW. 2002. γ-Radiation Influences Browning, Antioxidant Activity, and Malondialdehyde Level of Apple Juice. Journal of Agricultural and Food Chemistry 50: 710-715.

Feng G, Churey JJ, Worobo RW. 2007. Thermal inactivation of *Salmonella* and *Escherichia coli* O157:H7 on Alfalfa Seeds. Journal of Food Protection 70: 1698-1703.

Fichet G, Antloga K, Comoy E, Deslys JP, McDonnell G. 2007. Prion inactivation using a new gaseous hydrogen peroxide sterilisation process. Journal of Hospital Infection 67: 278-286.

Finch GR, Yuen WC, Uibel BJ. 1992. Inactivation of *Escherichia coli*

using ozone and ozone – hydrogen peroxide. Environmental Technology 13: 571-578.

Fisher CW, Lee DH, Dodge BA, Hamman KM, Robbins JB, Martin SE. 2000. Influence of catalase and superoxide dismutase on ozone inactivation of *Listeria monocytogenes*. Applied and Environmental Microbiology 66: 1405–1409

Foster JW, Hall HK. 1990. Adaptive Acidification Tolerance Response of *Salmonella typhimurium*. Journal of Bacteriology 172: 771-778.

Gabriel AA. 2012. Influences of heating temperatures, pH, and soluble solids on the decimal reduction times of acid-adapted and non-adapted *Escherichia coli* O157:H7 (HCIPH96055) in a defined liquid heating medium. International Journal of Food Microbiology 160: 50-57.

Gabriel AA, Nakano H. 2009. Inactivation of *Salmonella*, *E. coli* and *Listeria monocytogenes* in phosphate-buffered saline and apple juice by ultraviolet and heat treatments. Food control 20: 443-446.

Gachovska TK, Kumar S, Thippareddi H, Subbiah J, Williams F. 2008. Ultraviolet and pulsed electric field treatments have additive effect on

inactivation of *E. coli* in apple juice. Journal of food science 73: M412-M417.

Gao M, Tang J, Villa-Rojas R, Wang Y, Wang S. 2011. Pasteurization process development for controlling *Salmonella* in in-shell almonds using radio frequency energy. Journal of Food Engineering 104: 299-306.

García D, Hassani M, Mañas P, Condón S, Pagán R. 2005. Inactivation of *Escherichia coli* O157:H7 during the storage under refrigeration of apple juice treated by pulsed electric fields. Journal of Food Safety 25: 30-42.

Geeraerd AH, Herremans CH, Van Impe JF. 2000. Structural model requirements to describe microbial inactivation during a mild heat treatment. International Journal of Food Microbiology 59: 185-209.

Geeraerd AH, Valdramidis VP, Van Impe JF. 2005. GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. International Journal of Food Microbiology 102: 95-105.

Gieraltowski L, Julian E, Pringle J, Macdonald K, Quilliam D, Marsden-Haug N, Saathoff-Huber L, Von Stein D, Kissler B, Parish

M, Elder D, Howard-King V, Besser J, Sodha S, Loharikar A, Dalton S, Williams I, Barton Behravesh C. 2010. Nationwide outbreak of *Salmonella* Motevideo infections associated with contaminated imported black and red pepper: warehouse membership cards provide critical clues to identify the source. Epidemiology and Infection 141: 1244-1252.

Glaze WH. 1987. Drinking-water treatment with ozone. Environmental Science and Technology 21: 224-230.

Goepfert JM, Iskander IK, Amundson CH. 1970. Relation of the Heat Resistance of Salmonellae to the Water Activity of the Environment. Applied Microbiology 19: 429-433.

Goldblith SA, Wang DIC. 1967. Effect of Microwaves on *Escherichia coli* and *Bacillus subtilis*. Applied Microbiology 15: 1371-1375.

Gordon G, Bubnis B. 2002. Residual ozone measurement: Indigo sensitivity coefficient adjustment. Ozone: Science & Engineering 24: 17-28.

Goverd KA, Beech FW, Hobbs RP, Shannon R. 1979. The Occurrence and Survival of Coliforms and Salmonellas in Apple Juice and Cider.

Journal of Applied Bacteriology 46: 521-530.

Graham DM. 1997. Use of ozone for food processing. Food Technology 5: 72–75.

Grasso EM, Somerville JA, Balasubramaniam VM, Lee K. 2010. Minimal effects of high-pressure treatment on *Salmonella enterica* serovar Typhimurium inoculated into peanut butter and peanut products. Journal of Food Science 75: E522-E526.

Guzel-Seydim ZB, Greene AK, Seydim AC. 2004. Use of ozone in the food industry. LWT – Food Science and Technology 37: 453-460.

Ha JW, Kang DH. 2015. Combining Lactic Acid Spray with Near-Infrared Radiant Heating To Inactivate *Salmonella enterica* Serovars Enteritidis on Almonds and Pine Nut Kernels. Applied and Environmental Microbiology 81: 4517-4524.

Ha JW, Kang DH. 2013a. Simultaneous Near-Infrared Radiant Heating and UV Radiation for Inactivating *Escherichia coli* O157:H7 and *Salmonella enterica* Serovar Typhimurium in Powdered Red Pepper (*Capsicum annuum* L.). Applied and Environmental Microbiology 79:

6568-6575.

Ha JW, Kim SY, Ryu S, Kang DH. 2013b. Inactivation of *Salmonella enterica* serovar Typhimurium and *Escherichia coli* O157:H7 in peanut butter cracker sandwiches by radio-frequency heating. Food Microbiology 34: 145-150.

Harris LJ, Uesugi AR, Abd SJ, McCarthy KL. 2012. Survival of *Salmonella* Enteritidis PT 30 on inoculated almond kernels in hot water treatments. Food Research International 45: 1093-1098.

He Y, Guo D, Yang J, Tortorello ML, Zhang W. 2011. Survival and Heat Resistance of *Salmonella enterica* and *Escherichia coli* O157:H7 in Peanut Butter. Applied and Environmental Microbiology 77: 8434-8438.

He Y, Li Y, Salazar JK, Yang J, Tortorello ML, Zhang W. 2013. Increased water activity reduces the thermal resistance of *Salmonella enterica* in peanut butter. Applied and Environmental Microbiology 79: 4763-4767.

Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. 1993. Dietary antioxidant flavonoids and risk of coronary heart disease:

the Zutphen Elderly Study. Lancet 342: 1007-1011.

Hertwig C, Reineke K, Ehlbeck J, Erdoğdu B, Rauh C, Schlüter O. 2015a. Impact of remote plasma treatment on natural microbial load and quality parameters of selected herbs and spices. Journal of Food Engineering 167: 12-17.

Hertwig C, Reineke K, Ehlbeck J, Knorr D, Schlüter O. 2015b. Decontamination of whole black pepper using different cold atmospheric pressure plasma applications. Food Control 55: 221-229.

Hohmann EL. 2001. Nontyphoidal Salmonellosis. Clinical Infectious Disease 32: 263-269.

Hong EJ. 2016. Inactivating *Salmonella* Typhimurium on Contaminated Alfalfa Seeds for Sprouting without Detrimental Effect on Viability of Seeds. Master Thesis, Seoul National University, 74 pp.

Hong EJ, Kang DH. 2016. Effect of sequential dry heat and hydrogen peroxide treatment on inactivation of *Salmonella* Typhimurium on alfalfa seeds and seeds germination. Food Microbiology 53: 9-14.

Hulme HE, Meikle LM, Wessel H, Strittmatter N, Swales J, Thompson

C, Nilsson A, Nibbs RJB, Milling S, Andren PE, Mackay CL, Dexter A, Bunch J, Goodwin RJA, Burchmore R, Wall DM. 2017. Mass spectrometry imaging identifies palmitoylcarnitine as an immunological mediator during *Salmonella* Typhimurium infection. Scientific Reports 7: 2786.

Hvizdzak AL, Beamer S, Jaczynski J, Matak KE. 2010. Use of Electron Beam Radiation for the Reduction of *Salmonella enterica* Serovars Typhimurium and Tennessee in Peanut Butter. Journal of Food Protection 73: 353-357.

Isanga J, Zhang G. 2007. Biologically Active Components and Nutraceuticals in Peanuts and Related Products: Review. Food Reviews International 23: 123-140.

Ishibashi Y, Yamamoto K, Tawaratsumida T, Yuasa T, Iwaya-Inoue M. 2008. Hydrogen peroxide scavenging regulates germination ability during wheat (*Triticum aestvum* L.) seed maturation. Plant Signaling and Behavior 3: 183-188.

Issacs S, Aramini J, Ciebin B, Farrar JA, Ahmed R, Middleton D,

Chandran AU, Harris LJ, Howes M, Chan E, Pichette AS, Campbell K, Gupta A, Lior LY, Pearch M, Clark C, Rodgers F, Jamieson F, Brophy I, Ellis A. 2005. An International Outbreak of Salmonellosis Associated with Raw Almonds Contaminated with Rare Phage Type of *Salmonella* Enteritidis. Journal of Food Protection 68: 191-198.

Jaquette CB, Beuchat LR, Mahon BE. 1996. Efficacy of Chlorine and Heat Treatment in Killing *Salmonella stanley* Inoculated onto Alfalfa Seeds and Growth and Survival of the Pathogen during Sprouting and Storage. Applied and Environmental Microbiology 62: 2212-2215.

Jayasena DD, Kim HJ, Yong HI, Park S, Kim K, Choe W, Jo C. 2015. Flexible thin-layer dielectric barrier discharge plasma treatment of pork butt and beef loin: Effects on pathogen inactivation and meat-quality attributes. Food Microbiology 46: 51-57.

Jeong S, Marks BP, Orta-ramirez A. 2009. Thermal Inactivation Kinetics for *Salmonella* Enteritidis PT30 on Almonds Subjected to Moist-Air Convection Heating. Journal of Food Protection 72: 1602-1609.

Jeong SG, Baik OD, Kang DH. 2017. Evaluation of radio-frequency

heating in controlling *Salmonella enterica* in raw shelled almonds. International Journal of Food Microbiology 254: 54-61.

Jeong SG, Kang DH. 2014. Influence of moisture content on inactivation of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in powdered red and black pepper spices by radio-frequency heating. International Journal of Food Microbiology 176, 15-22.

Jiao Y, Tang J, Wang S. 2014. A new strategy to improve heating uniformity of low moisture foods in radio frequency treatment for pathogen control. Journal of Food Engineering 141: 128-138.

Jordan SL, Pascual C, Bracey E, Mackey BM. 2001. Inactivation and injury of pressured-resistant strains of *Escherichia coli* O157 and *Listeria monocytogenes* in fruit juices. Journal of Applied Microbiology 91: 463-469.

Kahle K, Kraus M, Richling E. 2005. Polyphenol profiles of apple juices. Molecular Nutrition and Food Research 49: 797-806.

Kanner J. 1994. Oxidative processes in Meat and Meat Products: Quality Implications. Meat Science 36: 169-189.

Kang JW, Kang DH. 2016. Enhanced antimicrobial effect of organic acid washing against foodborne pathogens on broccoli by vacuum impregnation. International Journal of Food Microbiology 217: 85 -93.

Kaper JB, Nataro JP, Mobley HLT. 2004. Pathogenic *Escherichia coli*. Nature Reviews Microbiology 2: 123-140.

Kapperud G, Gustavsen S, Hellesnes I, Hansen AH, Lassen J, Hirn J, Jahkola M, Montenegro MA, Helmuth R. 1990. Outbreak of *Salmonella typhimurium* Infection Traced to Contaminated Chocolate and Caused by a Strain Lacking the 60-Megadalton Virulence Plasmid. Journal of Clinical Microbiology 28: 2597-2601.

Karagöz I, Moreira RG, Castell-Perez ME. 2014. Radiation D_{10} values for *Salmonella* Typhimurium LT2 and an *Escherichia coli* cocktail in pecan nuts (Kanza cultivar) exposed to different atmospheres. Food Control 39: 146-153.

Kenney SJ, Beuchat LR. 2004. Survival, Growth, and Thermal Resistance of *Listeria monocytogenes* in Products containing Peanut and Chocolate. Journal of Food Protection 67: 2205-2211.

Keyser M, Műller IA, Cilliers FP, Nel W, Gouws PA. 2008. Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. Innovative Food Science and Emerging Technologies 9: 348-354.

Khadre MA, Yousef AE, Kim JG. 2001. Microbiological aspects of ozone applications in food: A Review. Journal of food science 66: 1242-1252.

Kim C, Hung YC, Brackett RE, Lin CS. 2003. Efficacy of Electrolyzed Oxidizing Water in Inactivating *Salmonella* on Alfalfa Seeds and Sprouts. Journal of Food Protection 66: 208-214.

Kim HJ, Feng H, Kushad MM, Fan X. 2006. Effects of Ultrasound, Irradiation, and Acidic Electrolyzed Water on Germination of Alfalfa and Broccoli Seeds and *Escherichia coli* O157:H7. Journal of Food Science 71: M168-M173.

Kim JE, Lee DU, Min SC. 2014. Microbial decontamination of red pepper powder by cold plasma. Food Microbiology 38: 128-136.

Kim JG, Yousef AE, Chism GW. 1999a. Use of ozone to inactivate microorganisms on lettuce. Journal of Food Safety 19: 17-34.

Kim JG, Yousef AE, Dave S. 1999b. Application of ozone for enhancing the microbiological safety and quality of foods: A review. Journal of Food Protection 62: 1071–1087.

Kimber MA, Kaur H, Wang L, Danyluk MD, Harris LJ. 2012. Survival of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on Inoculated Almonds and Pistachios Stored at -19, 4, and 24°C. Journal of Food Protection 75: 1394-1403.

Klein HG. 2005. Pathogen inactivation technology: cleansing the blood supply. Journal of Internal Medicine 257: 224-237.

Krapfenbauer G, Kinner M, Gössinger M, Schönlechner R, Berghofer E. 2006. Effect of Thermal Treatment on the Quality of Cloudy Apple Juice. Journal of Agricultural and Food Chemistry 54: 5453-5460.

Labatiuk CW, Belosevic M, Finch GR. 1994. Inactivation of *Giardia muris* Using Ozone and Ozone – Hydrogen Peroxide. Ozone: Science and Engineering 16: 67-78.

Lacombe A, Niemira BA, Sites J, Boyd G, Gurtler JB, Tyrell B, Fleck M. 2016. Reduction of Bacterial Pathogens and Potential Surrogates on

the Surfaces of Almonds Using High-Intensity 405-Nanometer Light. Journal of Food Protection 79: 1840-1845

Landry KS, Chang Y, McClements DJ, McLandsborough L. 2014. Effectiveness of a novel spontaneous carvacrol nanoemulsion against *Salmonella enterica* Enteritidis and *Escherichia coli* O157:H7 on contaminated mung bean and alfalfa seeds. International Journal of Food Microbiology 187: 15-21.

Lee KW, Kim YJ, Kim DO, Lee HJ, Lee CY. 2003. Major Phenolics in Apple and Their Contribution to the Total Antioxidant Capacity. Journal of Agricultural and Food Chemistry 51: 6516-6520.

Lee SH, Choi W, Jun S. 2016. Conventional and Emerging Combination Technologies for Food Processing. Food Engineering Review 8: 414-434.

Lee SY, Sagong HG, Ryu S, Kang DH. 2012. Effect of continuous ohmic heating to inactivate *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in orange juice and tomato juice. Journal of Applied Microbiology 112: 723–731.

Leizerson S, Shimoni E. 2005. Stability and Sensory Shelf Life of Orange Juice Pasteurized by Continuous Ohmic Heating. Journal of Agricultural and Food Chemistry 53: 4012-4018.

Leontowicz H, Gorinstein S, Lojek A, Leontowicz M, Ciz M, Soliva-Fortuny R, Park YS, Jung ST, Trakhtenberg S, Martin-Belloso O. 2002. Comparative content of some bioactive compounds in apples, peaches and pears and their influence on lipids and antioxidant capacity in rats. The Journal of Nutritional Biochemistry 13: 603-610.

Li C, Huang L, Chan J. 2014. Comparative study of thermal inactivation kinetics of *Salmonella* spp. in peanut butter and peanut butter spread. Food Control 45: 143-149.

Liao H, Hu X, Liao X, Chen F, Wu J. 2007. Inactivation of *Escherichia coli* inoculated into cloudy apple juice exposed to dense phase carbon dioxide. International Journal of Food Microbiology 118: 126–131.

Limcharoenchat P, Marks BP, Jeong S. 2014. The effect of Changing Almond Water Activity on Thermal Inactivation of *Salmonella* Enteritidis PT30 during Drying Heating. ASABE paper No. 141913989. St. Joseph,

Mich.: ASABE.

Lin J, Smith MP, Chapin KC, Baik HS, Bennett GN, Foster JW. 1996. Mechanisms of Acid Resistance in Enterohemorrhagic *Escherichia coli*. Applied and Environmental Microbiology 62: 3094-3100.

Little CL, Omotoye R, Mitchell RT. 2003. The microbiological quality of ready-to-eat foods with added spices. International Journal of Environmental Health Research 13: 31-42.

Luo C, Zhang Y, Ding Y, Shan Z, Chen S, Yu M, Hu FB, Liu L. 2014. Nut consumption and risk of type 2 diabetes, cardiovascular disease, and all-cause mortality: a systemic review and meta-analysis. The American Journal of Clinical Nutrition 100: 256-269.

Lyytikäinen O, Autio T, Maijala R, Ruutu P, Honkanen-Buzalski T, Miettinen M, Hatakka M, Mikkola J, Anttila VJ, Johansson T, Rantala L, Aalto T, Korkeala H, Siitonen A. 2000. An Outbreak of *Listeria monocytogenes* Serotype 3a Infections from Butter in Finland. Journal of Infectious Diseases 181: 1838-1841.

Ma L, Zhang G, Gerner-Smidt P, Mantripragada V, Ezeoke I, Doyle

MP. 2009. Thermal Inactivation of *Salmonella* in Peanut Butter. Journal of Food Protection 72: 1596-1601.

Mafart P, Couvert O, Gaillard S, Leguerinel I. 2002. On calculating sterility in thermal preservation methods: application of the Weibull frequency distribution model. International Journal of Food Microbiology 72: 107-113.

Malik DJ, Shaw CM, Shama G, Clokie MRJ, Rielly CD. 2016. An Investigation into the Inactivation Kinetics of Hydrogen Peroxide Vapor Against *Clostridium difficile* Endospores. Chemical Engineering Communications 203: 1615-1624.

Mattick KL, Jørgensen F, Legan JD, Lappin-Scott HM, Humphrey TJ. 2000. Habituation of *Salmonella* spp. at Reduced Water Activity and Its Effect on Heat Tolerance. Applied and Environmental Microbiology 66: 4921-4925.

Mattick LR, Moyer JC. 1983. Composition of apple juice. Journal-Association Official Analytical Chemists. 66: 1251-1255.

Mazzoni AM, Sharma RR, Demirci A, Ziegler GR. 2001. Supercritical

carbon dioxide treatment to inactivate aerobic microorganisms on alfalfa seeds. Journal of Food Safety 21: 215-223.

Mazzotta AS. 2001. Thermal Inactivation of Stationary-Phase and Acid-Adapted *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in Fruit Juices. Journal of Food Protection 64: 315-320.

McDonnell G, Russell AD. 1999. Antiseptics and disinfectant: activity action, and resistance. Clinical Microbiology Review 12: 147-179.

McWatters KH, Chinnan MS, Walker SL, Doyle MP, Lin CM. 2002. Consumer Acceptance of Fresh-Cut Iceberg Lettuce Treated with 2% Hydrogen Peroxide and Mild Heat. Journal of Food Protection 65: 1221-1226.

Mexis SF, Badeka AV, Kontominas MG. 2009. Quality evaluation of raw ground almond kernels (*Prunus dulcis*): Effect of active and modified atmosphere packaging, container oxygen barrier and storage conditions. Innovative Food Science and Emerging Technologies 10: 580-589.

Müller LL, Hjertqvist M, Payne L, Petterson H, Olsson A, Forshell LP, Adersson Y. 2007. Cluster of *Salmonella* Enteritidis in Sweden 2005 –

2006 – suspected source: Almonds. Eurosurveillance 12: 153-155.

Mullin J. 1995. Microwave processing. In G. W. Gould (Ed.), New methods of food preservation (pp. 112-134). Bishopbriggs, UK: Blackie Academic and Professional.

Mura C, Chung YS. 1990. In Vitro Transcription Assay of Ozonated T7 Phage DNA. Environmental and Molecular Mutagenesis 16: 44-47.

Murray RGE, Steed P, Elson HE. 1965. The location of the mucopeptide in sections of the cell wall of *Escherichia coli* and other gram-negative bacteria. Canadian Journal of Microbiology 11: 547-560.

Nam KC, Ahn DU. 2003. Combination of aerobic and vacuum packaging to control lipid oxidation and off-odor volatiles of irradiated raw turkey breast. Meat Science 63: 389-395.

National Advisory Committee on Microbiological Criteria for Foods, 1999a. Microbiological safety evaluations and recommendations on fresh produce. Food Control 10: 117-143.

National Advisory Committee on Microbiological Criteria for Foods, 1999b. Microbiological safety evaluations and recommendations on

sprout seeds. International Journal of Food Microbiology 52: 123-153.

Neetoo H, Chen H. 2011. Individual and combined application of dry heat with high hydrostatic pressure to inactivate *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. Food Microbiology 28: 119-127.

Newkirk J. 2016. Inactivition of *Salmonella enterica* and *Enterococcus faecium* on whole black peppercorns and cumin seeds using steam and ethylene oxide fumigation. M.S. thesis Virginia Tech, Blacksburg, 78 pp.

Niemira BA. 2012. Cold Plasma Reduction of *Salmonella* and *Escherichia coli* O157:H7 on Almonds Using Ambient Pressure Gases. Journal of Food Science 77: M171-M175.

Ooi ST, Lorber B. 2005. Gastroenteritis Due to *Listeria monocytogenes*. Clinical Infectious Diseases 40: 1327-1332.

Oularbi S, Mansouri B. 1996. Decontamination of black pepper and red pepper by gamma radiation. Radiation Physics and Chemistry 48: 386.

Oyarzábal OA, Nogueira MCL, Gombas DE. 2003. Survival of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in juice concentrates. Journal of Food Protection 66: 1595–1598.

Papadopoulou C, Demetriou D, Panagiou A, Levidiotou S, Gessouli H, Ioannides K, Antoniades G. 1995. Survival of Enterobacteria in liquid cultures during microwave radiation and conventional heating. Microbiological Research 150: 305-309.

Parish ME. 1997. Public Health and Nonpasteurized Fruit Juices. Critical Reviews in Microbiology 23: 109-119.

Patil S, Bourke P, Frias JM, Tiwari BK, Cullen PJ. 2009. Inactivation of *Escherichia coli* in orange juice using ozone. Innovative Food Science and Emerging Technologies 10: 551–557.

Patil S, Torres B, Tiwari BK, Wijngaard HH, Bourke P, Cullen PJ, O' Donnell CP, Valdramidis VP. 2010a. Safety and quality assessment during the ozonation of cloudy apple juice. Journal of Food Science 75: M437-M443.

Patil S, Valdramidis VP, Cullen PJ, Frias J, Bourke P. 2010b. Inactivation of *Escherichia coli* by ozone treatment of apple juice at different pH levels. Food Microbiology 27: 835-840.

Paul Ross R, Morgan S, Hill C. 2002. Preservation and fermentation: past,

present and future. International Journal of Food Microbiology 79: 3-16.

Prentice GA, Clegg LFI. 1974. The effect of incubation temperature on the recovery of spores of *Bacillus subtilis* 2057. Journal of Applied Bacteriology 37: 501-508.

Pucciarelli AB, Benassi FO. 2005. Inactivation of *Salmonella* Enteritidis on Raw poultry Using Microwave Heating. Brazilian Archives of Biology and Technology 48: 939-945.

Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. 2005. Epidemiology of *Escherichia coli* O157:H7 Outbreaks, United States, 1982-2002. Emerging Infectious Diseases 11: 603-609.

Reiss G, Kunz P, Koin D, Keeffe EB. 2006. *Escherichia coli* O157:H7 Infection in Nursing Homes: Review of Literature and Report of Recent Outbreak. Journal of American Geriatrics Society 54: 680-684.

Rico CW, Kim GR, Ahn JJ, Kim HK, Furuta M, Kwon JH. 2010. The comparative effect of steaming and irradiation on the physicochemical and microbiological properties of dried red pepper (*Capsicum annum* L.). Food Chemistry 119: 1012-1016.

Riveros CG, Mestrallet MG, Gayol MF, Quiroga PR, Nepote V, Grosso NR. 2010. Effect of storage on chemical and sensory profiles of peanut pastes prepared with high-oleic and normal peanuts. Journal of the Science of Food and Agriculture 90: 2694-2699.

Robertson K, Green A, Allen L, Ihry T, White P, Chen WS, Douris A, Levine J. 2016. Foodborne Outbreaks Reported to the U.S. Food Safety and Inspection Service, Fiscal Years 2007 through 2012. Journal of Food Protection 79: 442-447.

Rushdy AA, Stuart JM, Ward LR, Bruce J, Threlfall EJ, Punia P, Bailey JR., 1998. National outbreak of *Salmonella* senftenberg associated with infant food. Epidemiology and Infection 120: 125-128.

Rutalta WA, Weber DJ. 2016. Disinfetion, sterilization, and antisepsis: An overview. American Journal of Infection Control 44: e1-e6.

Ryu JH, Beuchat LR. 1998. Influence of acid tolerance responses on survival, growth, and thermal cross-protection of *Escherichia coli* O157:H7 in acidified media and fruit juices. International Journal of Food Microbiology 45: 185-193.

Salamina G, Dalle Donne E, Niccolini A, Poda G, Cesaroni D, Bucci M, Fini R, Maldini M, Schuchat A, Swaminathan B, Bibb W, Rocourt J, Binkin N, Salmaso S. 1996. A foodborne outbreak of gastroenteritis involving *Listeria monocytogenes*. Epidemiology and Infection 117: 429-436.

Sauer A, Moraru CI. 2009. Inactivation of *Escherichia coli* ATCC 25922 and *Escherichia coli* O157:H7 in Apple Juice and Apple Cider, Using Pulsed Light Treatment. Journal of Food Protection 72: 937-944.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM. 2011. Foodborne Illness Acquired in the United States-Major Pathogens. Emerging Infectious Diseases 17: 7-15.

Schnabel U, Niquet R, Krohmann U, Polak M, Schlüter O, Weltmann K. D, Ehlbeck J. 2012. Decontamination of Microbiologically Contaminated Seeds by Microwave Driven Discharge Processed Gas. Journal of Agricultural Science and Applications 1: 99-105.

Scott DBM, Lesher EC. 1963. Effect of Ozone on Survival and Permeability of *Escherichia coli*. Journal of Bacteriology 85: 567-576.

Scouten AJ, Beuchat LR. 2002. Combined effects of chemical, heat and ultrasound treatments to kill *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. Journal of Applied Microbiology 92: 668-674.

Shachar D, Yaron S. 2006. Heat Tolerance of *Salmonella enterica* Serovar Agona, Enteritidis, and Typhimurium in Peanut Butter. Journal of Food Protection 69: 2687-2691.

Shamila-Syuhada AK, Chuah LO, Wan-Nadiah WA, Cheng LH, Alkarkhi AFM, Effarizah ME, Rusul G. 2016. Inactivation of microbiota and selected spoilage and pathogenic bacteria in milk by combinations of ultrasound, hydrogen peroxide, and active lactoperoxidase system. International Dairy Journal 61: 120-125.

Sharma M, Adler BB, Harrison MD, Beuchat LR. 2005. Thermal tolerance of acid-adapted and unadapted *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes* in cantaloupe juice and watermelon juice. Letters in Applied Microbiology 41: 448-453.

Sharma RR, Demirci A. 2003. Inactivation of *Escherichia coli* O157:H7 on Inoculated Alfalfa Seeds with Pulsed Ultraviolet Light and Response

Surface Modeling. Journal of Food Science 68: 1448-1453.

Sharma RR, Demirci A, Beuchat LR, Fett WF. 2002. Inactivation of *Escherichia coli* O157:H7 on Inoculated Alfalfa Seeds with Ozonated Water and Heat Treatment. Journal of Food Protection 65: 447-451.

Shamis Y, Taube A, Shramkov Y, Mitik-Dineva N, Vu B, Ivanova EP. 2008. Development of a Microwave Treatment Technique for Bacterial Decontamination of Raw Meat. International Journal of Food Engineering 4: 8

Sharma RR, Demirci A, Beuchat LR, Fett WF. 2002. Inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds with ozonated water and heat treatment. Journal of Food Protection 65: 447–451.

Simmons GF, Smilanick JL, John S, Margosan DA. 1997. Reduction of Microbial Populations on Prunes by Vapor-Phase Hydrogen Peroxide. Journal of Food Protection 60: 188-191.

Singleton V, Rossi J. 1965. Colorimetry of total phenols with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology Viticulture 16: 144-158.

Sivapalasingam S, Friedman CR, Cohen L, Tauxe RV. 2004 Fresh Produce : A Growing Cause of Outbreaks of Foodborne Illness in the United States, 1973 through 1997. Journal of Food Protection 67: 2342-2353.

Song WJ, Kang DH. 2016a. Inactivation of *Salmonella* Senftenberg, *Salmonella* Typhimurium and *Salmonella* Tennessee in peanut butter by 915 MHz microwave heating. Food Microbiology 53: 48-52.

Song WJ, Kang DH. 2016b. Influence of water activity on inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in peanut butter by microwave heating. Food Microbiology 60: 104-111.

Song WJ, Sung HJ, Kang DH. 2014a. Inactivation of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in apple juices with different soluble solids content by combining ozone treatment with mild heat. Journal of Applied Microbiology 118: 112-122.

Song WJ, Sung HJ, Kim SY, Kim KP, Ryu S, Kang DH. 2014b. Inactivation of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium

in black pepper and red pepper by gamma irradiation. International Journal of Food Microbiology 172: 125-129.

Staehelin J, Hoigné J. 1985. Decomposition of ozone in water in the presence of organic solutes acting as promoters and inhibitors of radical chain reactions. Environmental Science and Technology 19: 1206-1213.

Steele BT, Murphy N, Arbus GS, Rance CP. 1982. An outbreak of hemolytic uremic syndrome associated with ingestion of fresh apple juice. The Journal of Pediatrics 101: 963-965.

Steenstrup LD, Floros JD. 2004 Inactivation of *Escherichia coli* O157:H7 in Apple Cider by Ozone at various Temperatures and Concentrations. Journal of Food Processing and Preservation 28: 103-116.

Stephens PJ, Druggan P, Nebe-von Caron G. 2000. Stressed *Salmonella* are exposed to reactive oxygen species from two independent sources during recovery in conventional culture media. International Journal of Food Microbiology 60: 269-285.

Strickland AF, Perkins WS. 1995 Decolorization of Continuous Dyeing Wastewater by Ozonation. Textile Chemist and Colorist 27: 11-15.

Sung HJ, Kang DH. 2014. Effects of a 915 MHz microwave system on inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in salsa. LWT – Food Science Technology 59: 754-759.

Sung HJ, Song WJ, Kim KP, Ryu S, Kang DH. 2014 Combination effect of ozone and heat treatment for the inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in apple juice. International Journal of Food Microbiology 171: 147-153.

Svoboda A, Shaw A, Dzubak J, Mendonca A, Wilson L, Nair A. 2016. Effectiveness of Broad-Spectrum Chemical Produce Sanitizers against Foodborne Pathogens as In Vitro Planktonic Cells and on the Surface of Whole Cantaloupes and Watermelons. Journal of Food Protection 79: 524-530.

Tanaka F, Mallikarjunan P, Hung YC. 1999. Dielectric properties of shrimp related to microwave frequencies: from frozen to cooked stage. Journal of Food Process Engineering 22: 455-468.

Taormina PJ, Beuchat LR. 1999. Comparison of Chemical Treatments to

Eliminate Enterohemorrhagic *Escherichia coli* O157:H7 on Alfalfa Seeds. Journal of Food Protection 62: 318-324.

Teo AY, Ravishankar S, Sizer CE. 2001 Effect of Low-Temperature, High-Pressure Treatment on the Survival of *Escherichia coli* O157:H7 and *Salmonella* in Unpasteurized Fruit Juices. Journal of Food Protection 64: 1122-1127.

Thanomsub B, Anupunpisit V, Chanphetch S, Watcharachaipong T, Poonkhum R, Srisukonth C. 2002. Effects of ozone treatment on cell growth and ultrastructural changes in bacteria. The Journal of General and Applied Microbiology 48: 193-199.

Tiwari BK, Muthukumarappan K, O'donnell CP, Cullen P J. 2008. Kinetics of freshly squeezed orange juice quality changes during ozone processing. Journal of Agricultural and Food Chemistry 56: 6416–6422.

Tiwari BK, O'Donnell CP, Muthukumarappan K, Cullen PJ. 2009a. Anthocyanin and colour degradation in ozone treated blackberry juice. Innovative Food Science and Emerging Technologies 10: 70–75.

Tiwari BK, O'Donnell CP, Patras A, Brunton N, Cullen PJ. 2009b.

Effect of ozone processing on anthocyanins and ascorbic acid degradation of strawberry juice. Food Chemistry 113: 1119–1126.

Tiwari BK, O'Donnell CP, Patras A, Brunton N, Cullen PJ. 2009c Anthocyanins and color degradation in ozonated grape juice. Food and Chemical Toxicology 47: 2824-2829.

Toribio JL, Lozano JE. 1984 Nonenzymatic Browning in Apple Juice Concentrate during Storage. Journal of Food Science 49: 889-892.

Toribio JL, Lozano JE 1986. Heat Induced Browning of Clarified Apple Juice at High Temperature. Journal of Food Science **51**, 172-175.

Torlak E. 2014. Efficacy of ozone against *Alicyclobacillus acidoterrestris* spores in apple juice. International Journal Food Microbiology 172: 1-4.

Torres B, Tiwari BK, Patras A, Wijngaard HH, Brunton N, Cullen PJ, O'Donnell CP. 2011. Effect of ozone processing on the colour, rheological properties and phenolic content of apple juice. Food Chemistry 124: 721–726.

Trinetta V, Vaidya N, Linton R, Morgan M. 2011. A comparative study on the effectiveness of chlorine dioxide gas, ozone gas and e-beam

irradiation treatments for inactivation of pathogens inoculated onto tomato, cantaloupe and lettuce seeds. International Journal of Food Microbiology 146: 203-206.

U.S. Department of Agriculture, 2007. Almonds grown in California; outgoing quality control requirements. Fed. Regist. 72, 15021-15036.

US Department of Agriculture. 2013. U. S. Apple Statistics table 18, Apple juice and cider: Supply and Utilization in the United States, 1980/81 to date. Data set released May, 2012. Available at : http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1825

United States Department of Agriculture, National Agriculture statistics Service. 2010. Agricultural Statistics 2010. Available at : www.nass.usda.gov/Publications/Ag_Statistics/2010/ last accessed July 14, 2014.

US Food and Drug Administration (US FDA). 2001. Hazard Analysis and Critical Point (HACCP); Procedures for the safe and sanitary processing and importing of juice; Final rule. Federal Register 66, 33829-33830.

US Food and Drug Administration (US FDA). 2012. Direct food substances affirmed as generally recognized as safe: Ozone. 21CFR 184.1563. Food and Drug Administration, Washington, DC.

Vadivambal R, Jayas DS. 2010. Non-uniform Temperature Distribution During Microwave Heating of Food Materials – A Review. Food and Bioprocess Technology 3: 161-171.

Valero A, Cejudo M, García-Gimeno RM. 2014. Inactivation kinetics for *Salmonella* Enteritidis in potato omelet using microwave heating treatments. Food Control 43: 175-182.

Vega-Gálvez A, Lemus-Mondaca R, Bilbao-Sáinz C, Fito P, Andrés A. 2008. Effect of air drying temperature on the quality of rehydrated dried red bell pepper (var. Lamuyo). Journal of Food Engineering 85: 42-50.

Vojdani JD, Beuchat LR, Tauxe RV. 2008. Juice-associated outbreaks of human illness in the United States, 1995 through 2005. Journal of Food Protection 71: 356–364.

Vurma M, Pandit RB, Sastry SK, Yousef AE. 2009. Research Paper, Inactivation of *Escherichia coli* O157:H7 and natural microbiota on

spinach leaves using gaseous ozone during vacuum cooling and simulated transportation. Journal of Food Protection 72: 1538–1546.

Waje CK, Kim HK, Kim KS, Todoriki S, Kwon JH. 2008. Physicochemical and Microbiological Qualities of Steamed and Irradiated Ground Black Pepper (*Piper nigrum* L.). Journal of Agricultural and Food Chemistry 56: 4592-4596.

Wang S, Monzon M, Johnson JA, Mitcham EJ, Tang J. 2007. Industrial-scale radio frequency treatments for insect control in walnut Ⅱ: Insects mortality and product quality. Postharvest Biology and Technology 45: 247-253.

Wang Y, Wig TD, Tang J, Hallberg LM. 2003. Dielectric properties of foods relevant to RF and microwave pasteurization and sterilization. Journal of Food Engineering 57: 257-268.

Williams RC, Issacs S, Decou ML, Richardson EA, Buffett MC, Slinger RW, Brodsky MH, Ciebin BW, Ellis A, Hockin J. 2000. Illness outbreak associated with *Escherichia coli* O157:H7 in Genoa salami. Canadian Medical Association Journal 162: 1409-1413.

Williams RC, Sumner SS, Golden DA. 2004. Survival of *Escherichia coli* O157:H7 and *Salmonella* in apple cider and orange juice as affected by ozone and treatment temperature. Journal of Food Protection 67: 2381-2386.

Williams RC, Sumner SS, Golden DA. 2005. Inactivation of *Escherichia coli* O157:H7 and *Salmonella* in apple cider and orange juice treated with combinations of ozone, dimethyl dicarbonate, and hydrogen peroxide. Journal of Food Science 70: M197-M201.

Wood JP, Calfee MW, Clayton M, Griffin-Gatchalian N, Touati A, Ryan S, Mickelsen L, Smith L, Rastogi V. 2016. A simple decontamination approaches using hydrogen peroxide vapour for *Bacillus anthracis* spore inactivation. Journal of Applied Microbiology 121: 1603-1615.

Yang J. Bingol G. Pan Z. Brandl MT, McHugh TH, Wang H. 2010. Infrared heating for dry-roasting and pasteurization of almonds. Journal of Food Engineering 101: 273-280.

Yuste J, Fung DYC. 2004. Inactivation of *Salmonella* Typhimurium and

Escherichia coli O157:H7 in apple juice by a combination of nisin and cinnamon. Journal of Food Protection 67: 371-377.

Zhao J, Cranston PM. 1995. Microbial decontamination of black pepper by ozone and the effect of the treatment on volatile oil constituents of the spice. Journal of the Science of Food and Agriculture 68: 11-18.

Zheng XA, Han Z, Zi ZH. 2010. Effects of pulsed electric field treatments on quality of peanut oil. Food Control 21: 611-614.

Zhu J, Kuznetsov AV, Sandeep KP. 2007. Mathematical modeling of continuous flow microwave heating of liquids (effect of dielectric properties and design parameters). International Journal of Thermal Science 46: 328-341.

Zonta W, Mauroy A, Farnir F, Thiry E. 2016. Virucidal Efficacy of a Hydrogen Peroxide Nebulization Against Murine Norovirus and Feline Calicivirus, Two Surrogates of Human Norovirus. Food and Environmental Virology 8: 275-282.

국문초록

이 연구에서는 1) 가열처리를 통한 저 수분활성도 식품 내에 존재하는 식중독 균 저감화 효과 검증, 2) 비가열처리를 통한 식품 살균 효과 평가, 3) 비가열처리와 가열처리의 조합처리를 통한 저 수분활성도 식품 내의 병원균 제어 효과 규명을 목표로 하였다.

가열처리를 통한 저 수분활성도 식품 내에 존재하는 식중독 균 저감화 효과 검증을 위해 먼저 microwave 를 통한 살균 연구를 진행하였다. 땅콩 버터에 *S.* Senfterberg, *S.* Typhimurium, *S.* Tennessee 를 접종한 후, 915 MHz microwave (2, 4, 6 kW) 처리를 최대 5 분간 처리하였다. 6 kW 915 MHz microwave 5 분 처리 시에 3 가지 *Salmonella* 가 최소 3.24, 최대 4.26 log 저감화가 되었으며, 4 kW 915 MHz microwave 5 분 처리 시 1.14 에서 1.48 log 의 *Salmonella* 가 저감화된 것을 확인할 수 있었다. 또한 microwave 처리 후 품질변화를 확인하였을 때, 915 MHz microwave 처리는 땅콩버터의 산가, 과산화물가, 색을 변화시키지 않는 것을 확인해 볼 수 있었다.

다음으로는 땅콩버터의 수분활성도가 microwave 를 통한 의한

병원균 제어 효과에 미치는 영향을 확인하기 위하여 세가지 종류의 식중독 균이 접종된 땅콩버터의 수분활성도를 0.3, 0.4, 0.5 로 맞추어준 후 915 MHz microwave (2, 4, 6 kW) 처리를 진행하였다. 6 kW microwave 5 분 처리 시 세가지 식중독 균은 1.97 에서 5.17 log 이상 저감화되었으며, 4 kW microwave 5 분 처리 시 세가지 병원균이 0.41 에서 1.98 log 저감화된 것을 확인할 수 있었다. Microwave 를 통한 식중독 균의 저감화가 shouldering effect 를 보여 Weibull 모델과 Log-Linear + Shoulder 모델을 이용하여 식중독 균의 저감화 과정을 분석하여 보았을 때에 각각의 모델에 따라 계산된 미생물이 1/10 으로 감소되는데 걸리는 시간인 t_d 값은 수분활성도에 영향을 받지 않았으나, 미생물이 5 log 감소되는데 걸리는 시간인 t_{5d} 값의 경우 수분활성도가 증가할수록 감소하는 경향을 나타나는 것을 확인할 수 있었다.

마지막으로 microwave 의 주파수와 땅콩버터의 용량이 microwave 를 통한 땅콩버터 살균에 미치는 영향을 확인하기 위하여 50, 100, 200, 400 g 의 땅콩버터의 가운데 부분의 온도가 100℃ 가 될 때까지 915 와 2,450 MHz microwave 로 가열해주었다. 땅콩버터의 양이 증가할수록 2,450 MHz microwave 를 통한 가열에서는 처리 시간이 길어지는 경향이

나타났지만, 915 MHz microwave 의 경우 처리시간이 땅콩버터의 용량에 큰 영향을 받지 않는 것을 확인해 볼 수 있었다. 식중독균을 접종한 400 g 의 땅콩버터를 2,450 MHz microwave 로 최대 160 초, 915 MHz microwave 로 최대 90 초 처리해주었다. 2,450 MHz microwave 를 처리한 땅콩버터에서는 위치에 따라 식중독 균의 저감화 경향이 다르게 나타났고 재현성이 낮아 표준편차가 크게 나타난 반면 915 MHz microwave 를 처리한 땅콩버터에서는 이러한 경향이 나타나지 않았고, 식중독 균 또한 빠르게 저감화 되었으며 식품의 품질에 영향을 주지 않는 것을 확인할 수 있었다. 이에 따라 915 MHz microwave 가 2,450 MHz microwave 에 비하여 대용량의 샘플 살균에 적합한 기술임을 확인해 볼 수 있었다.

하지만 microwave 를 통한 살균 처리는 아몬드의 살균에는 적합하지 않아 아몬드의 살균에 적합한 처리 기술을 찾기 위한 연구를 진행하였다. 포장방법이 건열 처리를 통한 식중독 균 제어 효과에 미치는 영향을 평가하기 위하여 Open, Ambient-sealing, Vacuum-sealing 방식으로 *S.* Enteritidis PT 30, *S.* Typhimurium, *S.* Senftenberg 가 접종된 아몬드를 포장한 후, 건열 처리를 진행하였다. Vacuum-sealing 포장된 아몬드에서 식중독 균의 저감화가 가장 빨리 일어나는 것을 확인해볼 수

있었으며, Open 포장된 아몬드에서 가장 느린 저감화 속도를 확인할 수 있었다. 건열 처리는 아몬드의 색에 영향을 주지 않았으나 Ambient-sealing, Open 방식으로 포장 후 건열 처리를 진행한 아몬드의 수분함량과 수분활성도가 줄어든 것을 확인할 수 있었다. 이에 따라 진공포장 후 건열 처리 (vacuum-sealed dry heat)는 식품의 수분 증발을 막아 열전도율을 높여 효과적으로 식중독 균을 제어하는 것을 확인해 볼 수 있었다.

비 가열 처리를 통한 식품 내에 존재하는 식중독 균 제어 효과를 확인하기 위하여 오존과 pH의 조합처리를 이용한 사과주스 살균 연구를 진행하였다. pH 3.0, 4.0, 5.0 사과주스에 3가지의 식중독 균을 접종한 후 오존 가스 (유속: 3.0 l/min, 농도: 2.0-3.0 g/m^3) 처리를 최대 4분 동안 진행하였다. 4분 동안 오존을 처리해 준 결과 pH 3.0 사과주스에 존재하는 *E. coli* O157:H7이 검출한계 이하로 저감화되는 것을 확인할 수 있었다. 또한 pH 4.0과 5.0 사과주스에 4분동안 오존을 처리해준 결과 *E. coli* O157:H7이 5.12 log와 1.86 log 감소하는 것을 확인할 수 있었다. 오존과 낮은 pH의 조합처리는 매우 효과적으로 사과주스 내에 존재하는 식중독 균을 제어할 수 있었다. *S.* Typhimurium과 *L.*

*monocytogenes*의 경우에도 비슷한 경향을 나타내었다. 색의 경우 *b* 값을 제외하고는 유의적인 차이를 나타내지 않았으며 total phenolic contents도 오존 처리에 영향을 받지 않는 것을 확인해 볼 수 있었다.

Hydrogen peroxide vapor 의 효율을 더욱 높여주기 위하여 vacuumed hydrogen peroxide vapor 를 통한 저 수분활성도 식품 살균 효과를 확인해보았다. 식중독 균을 접종한 통후추와 고춧가루를 0.5 ml 의 10, 20, 30, 40, 50% 과산화수소를 이용하여 진공상태에서 과산화수소 증기를 1 분동안 처리해주었다. 50% vacuumed hydrogen peroxide vapor 처리는 후추에 존재하는 식중독 균을 4.52 ~ > 5.34 log 감소시켰으며 같은 처리는 고춧가루에 존재하는 식중독 균을 2.36 ~ 3.01 log 저감화시켰으며, 후추와 고춧가루의 색에 영향을 끼치지 않았다.

가열처리와 비 가열 처리의 조합처리를 통한 저 수분활성도 식품의 살균 효과를 확인하기 위하여 사과주스 농축액(12, 18, 36, 72 °Brix)에 오존처리 (유속: 3.0 l/min, 농도: 2.0−3.0 g/m^3)와 열처리 (25, 45, 50℃)를 동시에 20, 40, 60 초간 처리해주었다. 1 분간의 열처리 단독 처리 시에 사과주스에 존재하는 *E. coli* O157:H7 은 0 에서 4.18 log 저감화 되었다. 반면 1 분간의

조합처리(25, 45℃) 시 0.93 에서 3.87 log 저감화가 일어났고, 50℃ 에서 1 분간 진행한 조합처리는 *E. coli* O157:H 을 검출한계 이하로 감소시켰다. *S.* Typhimurium 의 경향성도 이와 비슷하였다. 오존과 열의 조합처리는 상승 효과를 나타내기도 하였다. 조합처리는 주스의 색에 영향을 주지 않았으며, 잔류 오존량도 허용 가능한 수준인 것을 확인할 수 있었다.

또 다른 조합처리인 Vacuumed hydrogen peroxide vapor 와 vacuum-sealed dry heat 의 조합처리를 통한 종자 내에 존재하는 식중독 균을 제어하는 연구를 진행하여 보았다. 병원균이 접종된 알팔파 종자를 30% 1 ml 의 vacuumed hydrogen peroxide vapor 처리를 진행한 후 개봉상태와 진공포장상태로 건열처리 (73℃)를 진행하였다. 증류수를 대조군으로 사용하였다. Vacuum-sealed dry heat 은 식중독 균을 효과적으로 저감화하는 것을 확인해 볼 수 있었지만, 건열 처리는 식중독 균을 크게 제어하지 못했다. 30% 1 ml 의 vacuumed hydrogen peroxide vapor 와 Vacuum-sealed dry heat 의 연속적인 처리는 병원균을 검출한계 이하로 제어할 수 있었으며 종자의 발아율에도 영향을 끼치지 않는 것을 확인해 볼 수 있었다.

본 연구를 통해 도출된 결과는 식품 산업에서 식중독 균을 제어하는 데에 활용될 수 있을 것이며 특히 저 수분활성도 식품의

살균에 도움이 될 수 있을 것이다. 가열, 비 가열, 가열과 비 가열처리의 조합처리는 현재 사용중인 살균 기술들의 대안으로 활용될 수 있을 것이다. 또한 이러한 식품의 살균연구, 특히 저 수분활성도 식품의 살균연구는 저 수분활성도 식품으로 인한 식중독을 줄여줄 수 있을 것이다.

주제어 : 식중독 균, 저감화, 저 수분활성도 식품, 가열처리, 비가열처리, 조합처리

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